Brian A. Whitton *Editor*

Ecology of Cyanobacteria II

Their Diversity in Space and Time

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Editor
Brian A. Whitton
School of Biological and Biomedical Sciences
Durham University
Durham
United Kingdom

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Preface

The publication in 2000 of *Ecology of Cyanobacteria. Their Diversity in Time and Space* aimed to assemble some of the most important information on ecology in the same way as *The Molecular Ecology of Cyanobacteria* edited by Donald Bryant had done in 1994 for other aspects of cyanobacteria. Malcolm Potts, who co-edited that volume, and I used the Preface to consider what would happen in ecology during the coming years. The impact of molecular studies on ecological understanding and commercial developments seemed likely to be especially important and this is what has happened. Many other discoveries of particular relevance for ecology have also been reported, such as advances in understanding about cyanobacterial nitrogen fixation and phosphorus acquisition in the oceans, and about the roles of extracellular polymers in many types of environment. A further book, *The Cyanobacteria. Molecular Biology, Genomics and Evolution* edited by Antonia Herrero and Enrique Flores and published in 2008, assembled a great deal of information on the non-ecological topics. It therefore seemed time for an entirely new book on ecology.

Ecology of Cyanobacteria II. Their Diversity in Space and Time gives this ecological account. Unfortunately, Malcolm Potts was too busy with projects and travel to join in the editing, although he has co-authored three chapters. Although almost half the quoted references have been published since the 2000 volume, all the authors provide a broad perspective on their subject and not just an account of recent advances. Molecular data enter into every chapter. There is also a lot about cyanobacterial biotechnology, but perhaps not quite as much as we thought there would be when writing in 2000. In no other group of organisms is it possible to see so clearly how ecology, physiology, biochemistry, ultrastructure and molecular biology interact. Hopefully this information can be put to many more practical uses during the coming decade. I am still looking forward to the day when a third title in the series is justified, *Practical Uses and Problems of Cyanobacteria*.

Some of the worries in 2000 still apply. The failure to consider older literature may not matter in the case of molecular data, but in ecology it often does. There is much of interest in pre-1980 literature, and modern understanding about a topic often makes it possible to extract a lot more from older accounts. A problem which seems to have become worse since 2000 is the tendency for many researchers to depend entirely on reading the abstract, if they do consult old literature at all. No doubt this is partly a matter of time, but the main reason is the ease with which most abstracts of long past literature can be obtained using the Internet and the difficulty many researchers have in accessing the full accounts in papers and books. However, the abstracts often omit the very aspects which are of most interest nowadays. Perhaps someone will advise me what should happen to the twenty or so thousand reprints of old papers in my garage.

A new worry is that Aldabra Atoll, about which the previous Preface enthused because of the diversity of its cyanobacterial communities, has become a challenge for researchers to visit due to the risk of hostage seizure in the Indian Ocean. Hopefully, some day soon it will again become possible for cyanobacteriologists to study this paradise.

My thanks to all the authors, and especially to those who allowed themselves to get involved in vigorous discussion about scientific topics. It was therefore very sad to learn at a late stage that Patrizia Albertano had died, particularly as she was corresponding about the proof just six days before this without ever mentioning her illness. I also much appreciate the support of Suzanne Mekking and Martine van Bezooijen at Springer and their enthusiasm for a new book with plenty of colour figures and additional online material. It was yet another sad occasion to learn in late February that Martine had died, but I am grateful to the other staff for helping to keep publication on schedule.

Brian A. Whitton
April 2012

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Contributors

David G. Adams Faculty of Biological Sciences, University of Leeds, Leeds, UK

Patrizia Albertano† Dipartimento di Biologia, Universita degli Studi di Roma "Tor Vergata", 00133 Rome, Italy

Roda Fahad Al-Thani Department of Biological and Environmental Sciences Department, College of Arts and Sciences, Qatar University, P.O. Box 2713, Doha, Qatar

Justin D. Brookes School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia

Cristiana Callieri National Research Council, Institute of Ecosystem Study, Largo Tonolli 50, 28922, Verbania Pallanza, Italy

Richard W. Castenholz Institute of Ecology and Evolutionary Biology, University of Oregon, Eugene, OR 97403-5289, USA

Martha R.J. Clokie Department of Infection, Immunity and Inflammation, University of Leicester, P.O. Box 138, Leicester, UK

Geoffrey A. Codd Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, UK

Gertrud Cronberg Aquatic Ecology, Ecology Building, University of Lund, BOX 65, S-22100, Lund, Sweden

Paula S. Duggan Faculty of Biological Sciences, University of Leeds, Leeds, UK

George G. Ganf School of Earth and Environmental Sciences, University of Adelaide, Adelaide, SA 5005, Australia

Kunshan Gao State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian 361005, PR China

Ferran Garcia-Pichel School of Life Sciences, Arizona State University, Tempe, AZ 85287-45, USA

Claudia B. Grewe Research and Development, Salata GmbH, An der Salzbrücke, 98617 Ritschenhausen, Germany

David P. Hamilton Environmental Research Institute, The University of Waikato, Hamilton, New Zealand

Richard F. Helm Department of Biochemistry, Life Sciences I, Virginia Tech, Blacksburg, VA 24061, USA

Chunxiang Hu State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China

† Deceased

Owen Jackson Faculty of Biological Sciences, University of Leeds, Leeds, UK

Nicholas H. Mann School of Life Sciences, University of Warwick, Coventry, UK

Amy M. Marcarelli Department of Biological Sciences, Michigan Technological University, Houghton, MI 49931, USA

Blahoslav Maršálek Centre for Cyanobacteria and their Toxins, Institute of Botany, Academy of Sciences of the Czech Republic, Brno, Brno CZ-65720, Czech Republic

Pilar Mateo Departamento de Biologia, Facultad de Ciencias, Universidad Autonoma de Madrid, 28049 Madrid, Spain

James S. Metcalf Institute for Ethnomedicine, Box 3464, Jackson WY 83001, USA

Scott R. Miller Division of Biological Sciences, The University of Montana, Missoula, MT, USA

Roderick L. Oliver CSIRO Land and Water, CSIRO, PMB2, Glen Osmond, SA 5064, Australia

Aharon Oren Division of Microbial Molecular Ecology, Alexander Silverman Institute of Life Sciences, Moshe Shilo Minerva Center for Marine Biogeochemistry, The Hebrew University, 91904, Jerusalem, Israel

Hans W. Paerl Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC 28557, USA

Allan Pentecost Freshwater Biological Association, Ambleside, Cumbria, UK

Malcolm Potts Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, P.O. Box 2713, Doha, Qatar

Otto Pulz Research and Development, Salata GmbH, An der Salzbrücke, 98617 Ritschenhausen, Germany

Antonio Quesada Departamento de Biologia, Facultad de Ciencias, Universidad Autonoma de Madrid, 28049 Madrid, Spain

John A. Raven Division of Plant Sciences, University of Dundee at TJHI, The James Hutton Institute, Invergowrie, Dundee, UK

David J. Scanlan School of Life Sciences, University of Warwick, Coventry, UK

J. William Schopf Department of Earth and Space Sciences, Center for the Study of Evolution and the Origin of Life, Institute of Geophysics and Planetary Physics, Molecular Biology Institute, University of California, Los Angeles, CA 90095-1567, USA

J. Thad Scott Department of Crop, Soil and Environmental Sciences, Dale Bumpers College of Agricultural, Food and Life Sciences, University of Arkansas, Fayetteville, AR 72701, USA

Lenka Šejnohová Institute of Botany, Academy of Sciences of the Czech Republic, Brno, Brno CZ-65720, Czech Republic

Claudio Sili Istituto per lo Studio degli Ecosistemi, CNR, 10, 50019, Sesto Fiorentino, Firenze, Italy

Lucas J. Stal Department of Marine Microbiology, Royal Netherlands Institute of Sea Research (NIOZ), P. O. Box 140, NL-4400 AC Yerseke , The Netherlands

John G. Stockner Eco-Logic Ltd., 2624 Mathers Avenue, West Vancouver, BC, Canada

Giuseppe Torzillo Istituto per lo Studio degli Ecosistemi, CNR, 10, 50019, Sesto Fiorentino, Firenze, Italy

Warwick F. Vincent Departement de Biologie, Université de Laval, Quebec City, QC, Canada

Avigad Vonshak Microalgal Biotechnology, The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Beersheba, 84990, Israel

David M. Ward Land Resources and Environmental Sciences Department, Montana State University, Bozeman, MT 59717, USA

Brian A. Whitton School of Biological and Biomedical Sciences, Durham University, Durham, UK

Online Article Contributors

(Additional to those who also contributed to main chapters)

Amha Belay Earthrise Nutritionals LLC, PO Box 270 Glendale CA 91204, USA

Luis Henrique Z. Branco Dept. Zoologia e Botanica - IBILCE - UNESP, R. Cristovao Colombo, S. J. Rio Preto (SP), Brasil

Paul A. Broady School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

Alan Donaldson Mill Cottage, Allenheads, Northumberland NE47 9HE, UK

Bryan Kennedy Environmental Protection Agency, John Moore Road, Castlebar, Co. Mayo, Ireland

Glenn B. McGregor Environment and Resource Sciences, Queensland Department of Environment and Resource Management, Indooroopilly Qld 4068, Australia

Faradina Merican School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

Martin O'Grady Inland Fisheries Ireland, Swords Business Campus, Swords, Co. Dublin, Ireland

John D. Wehr Fordham University, Louis Calder Center – Biological Field Station, Armonk, NY USA

Introduction to the Cyanobacteria

1

Brian A. Whitton and Malcolm Potts

Contents

Summary

Features of cyanobacteria are introduced for non-specialists by highlighting topics in the various chapters. Aspects where much more is known now than a decade ago are pointed out, such as the importance of cyanobacterial nitrogen fixation in the oceans. This is followed by an account of the recent molecular studies most relevant for ecologists, especially topics not mentioned elsewhere in the book. Several ecological subjects of current interest are discussed, including research which seems important, but has sometimes been overlooked. Topics mentioned include sensing the environment and other organisms and signalling between cyanobacterial cells and between cyanobacteria and other organisms, and methods for studying N and P. The authors air their views on past and present matters concerning cyanobacterial taxonomy, molecular biology and nomenclature. Finally, comments are made on practical topics such as the use of cyanobacteria for inoculating soils, barley straw to control blooms and the likely contribution of cyanobacteria to developments in algal biotechnology during the coming decade.

1.1 What Are Cyanobacteria?

The cyanobacteria are photosynthetic prokaryotes found in most, though not all, types of illuminated environment. They are also quantitatively among the most important organisms on Earth. A conservative estimate of their global biomass is 3×10^{14} g C or a thousand million tonnes (10^{15} g) wet biomass (Garcia-Pichel et al. 2003). They all synthesize chlorophyll *a* and typically water is the electron donor during photosynthesis, leading to the evolution of oxygen. Most produce the phycobilin pigment, phycocyanin, which gives the cells a bluish colour when present in sufficiently high concentration, and is responsible for the popular name, blue-green algae; in some cases the red accessory pigment, phycoerythrin, is formed as well. A few genera, however,

B.A. Whitton (✉)
School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK
e-mail: b.a.whitton@durham.ac.uk

M. Potts
Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, POB 2713, Doha, Qatar
e-mail: malcolm.p@qu.edu.qa

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produce neither, but form other accessory pigments. These include some ecologically very important members of the ocean plankton. Among these, *Prochlorococcus* was first reported as recently as 1988 by Chisholm et al., but is now realized to be of major importance in the oceans (Zwirglmaier et al. 2007). The fact that such a significant organism could be overlooked for so long should encourage readers to take a critical approach to the present literature on cyanobacteria. Zhang and Bryant (2011) did this for the notion that cyanobacteria have an incomplete tricarboxylic acid cycle, which persisted in the literature for more than four decades and even got into several prominent textbooks. In their account dispelling this, they note how the misinterpretation of negative results can have a powerful, long-lasting impact on a topic.

Although the cyanobacteria live in a diverse range of environments, a number of features often contribute to their success. The following short account indicates some of these, but more detailed information can be found in the Introduction by (Whitton and Potts in 2000) and the other chapters in the present book. The temperature optimum for many or most cyanobacteria is higher by at least several degrees than for most eukaryotic algae (Castenholz and Waterbury 1989), thus encouraging their success in warmer climates (Kosten et al. 2012). Tolerance of desiccation and water stress is widespread (Chaps. 12 and 18) and cyanobacteria are among the most successful organisms in highly saline environments (Chap. 15). Terrestrial forms often tolerate high levels of ultra-violet irradiation (Chap. 19), whereas the success of many planktonic forms is favoured by their ability to utilize light for photosynthesis efficiently at low photon flux densities (van Liere and Walsby 1982). Free sulphide is tolerated by some species at much higher levels than by most eukaryotic algae and H_2S is sometimes utilized as the electron donor during photosynthesis (Cohen et al. 1986). Photosynthetic CO_2 reduction can sometimes proceed efficiently at very low concentrations of inorganic carbon (Pierce and Omata 1988; Chap. 17). The ability to form gas vacuoles in some common freshwater plankton species and the marine *Trichodesmium*, and hence increase cell buoyancy, is an asset where the rate of vertical mixing of the water column is relatively low (Chaps. 6, 7 and 8).

The ability of many species to fix N_2 provides a competitive advantage when combined N concentrations are low (Chaps. 4 and 5). In most well-oxygenated terrestrial and freshwater environments this takes place inside the heterocyst (Wolk et al. 1994), a thick-walled cell often with a nodule of cyanophyin, a polymer of two amino-acids, at one or both ends of the cell. However, a number of cyanobacteria have physiological strategies which permit them to fix N_2 under well oxygenated conditions even without a heterocyst and this becomes more widespread under micro-oxic conditions (Chap. 4). This is of considerable importance in some ecosystems such as wetland rice fields. Although heterocystous cyanobacteria are important in the Baltic Sea, where salinity is much less than that of the oceans (typically about one-fifth), oceanic N_2 fixation mostly occurs without heterocysts. Because of the lack of heterocystous species it has only recently been realized that cyanobacteria are the main N_2 fixers in the oceans (Díez et al. 2008). In the filamentous *Trichodesmium* and *Katagnymene* this occurs in specialized cells called diazocytes (El-Shehawy et al. 2003), but it also occurs in a globally distributed unicellular cyanobacterium which does not form O_2 and can fix N_2 in the light (Zehr et al. 2008). Only a few unicellular cyanobacteria in the <1 μm cell size fraction were found to lack nitrogenase genes during a global survey of the oceans (Rusch et al. 2007).

There are many symbiotic associations which include cyanobacteria (Chap. 23) and in the majority of cases it is the ability of the cyanobacterium to fix N_2 and then transfer it to the partner which is a key factor in the relationship. Some of these symbiotic associations have a long geological record, whereas others depend on frequent reinfection by a compatible strain of a particular cyanobacterium, usually *Nostoc*. In some associations the cyanobacterium is intracellular and this includes two marine planktonic diatoms which have a heterocystous species (Chaps. 22, 23). However, the spheroid bodies inside another diatom, *Rhopalodia gibba*, are evolutionarily related to the free-living unicells mentioned above which fix N_2, but do not evolve O_2 (Bothe et al. 2011). Like several other blue-green structures inside eukaryotic cells, these are no longer capable of living independently.

Growth of cyanobacteria in many ecosystems is limited by the availability of P and the importance of P as a nutrient is considered in a number of chapters. Among various topics in Chap. 5 it is explained which parts of the oceans are mostly likely to be limited by N or by P and also the importance of considering the ratio between the two. N and P sources for picophytoplankton and their uptake are considered in Chap. 8 and the influence of N:P ratio on the occurrence of N_2-fixers in freshwaters in Chap. 9. Only a few data are available about what N and P concentrations are actually experienced by subaerial algae and virtually nothing about their periodicity (Chap. 10). However, Chap. 11 makes clear the importance of the P supply for biological soil crusts in semi-desert regions, which almost always include one or more N_2-fixers. Chapter 22 explains the changes in N:P supply during the growth cycle of Rivulariaceae and also how differing periodicities in P supply in various environments influence the success of particular genera and species. Although Chap. 23 focuses on the transfer of fixed N to the cyanobacterial partner in symbiotic associations, P transfer the other way is sometimes also important.

It has long been recognized that cyanobacteria in freshwaters and soils tend to be much more diverse and abundant at higher pH values. There are, however, a considerable number of records at lower pH values. For instance, heterocystous

forms (*Hapalosiphon* and/or *Tolypothrix*) are frequent in small pools at pH 4.1–4.5 in *Sphagnu*m-dominated regions of the Flow Country in N-E. Scotland (B.A.W., unpublished data). In general, heterocystous species seem to be the ones most successful at low pH values, so perhaps they can only compete effectively with eukaryotes in situations where nitrogen fixation gives a clear advantage. However, Steinberg et al. (1998) found populations of two filamentous cyanobacteria (*Oscillatoria*/*Limnothrix* and *Spirulina* spp.) at pH 2.9 in Lichtenuaer See, Lusatia, Germany; eukaryotic algae were almost absent at the time. The authors failed to find planktonic picocyanobacteria anywhere below pH 4.5. This is one of several reports of lakes in lignite-mining areas in Germany and Poland with pH values below 3.0, which mention narrow Oscillatoriaceae in their species lists, presumably based on preserved samples. *Lyngbya ochracea*, for instance, is listed by Koproskowa (1995). As some of the reports make only a brief mention of the cyanobacteria, more detailed studies are needed to confirm them.

Interactions with limestone are a feature of some cyanobacteria. One of the more intriguing aspects is the capacity of some strains (euendoliths) to bore directly into the carbonate substrate. Inhibition assays and gene expression analyses with *Mastigocoleus* BC008 showed that in the dissolution process the uptake and transport of Ca^{2+} is driven by P-type Ca^{2+} ATPases (Garcia-Pichel et al. 2010), a sophisticated mechanism unparalleled among bacteria. Much remains to be discovered about the extent to which nutrients are acquired by endolithic Stigonematales like *Brachytrichia* and *Mastigocoleus* from the surrounding rock or the outside environment (Sect. 10.3.2).

Chapter 21 describes how cyanophage are among the most abundant biological entities on the planet and how they influence community structure and biogeochemical cycling. Although the influence of some bacteria and protists on cyanobacteria was reported long before that of cyanophage, it is still difficult to generalize on their overall importance in cyanobacterial ecology compared with that of cyanophage.

Several ecological features of cyanobacteria have brought them to the attention of the general public. Some species form dense blooms and there are numerous accounts of the problems caused by these and the methods adopted to control the blooms. Worldwide there are fewer than 30 species which cause a real nuisance, yet it is still difficult to generalize about the ecological requirements of many of them. However, understanding about one genus, *Microcystis*, is increasing especially rapidly (van Gremberghe et al. 2011; Chap. 7). Research in recent years has been accelerated by concern about massive *Microcystis* blooms in several large lakes in China, especially L. Taihu (Fig. 6.11), which are the source of drinking water for millions of people. The most serious problem caused by these blooms is the presence of toxins, which are harmful to humans, other mammals and often other types of organism (Chap. 24). At least some populations of all freshwater bloom-forming species studied contain toxins, and toxins have also been reported from many other cyanobacteria, including the marine *Trichodesmium* (Kerbrat et al. 2011). It is fortunate that the strains of *Arthrospira* which are marketed as "Spirulina" are not toxic, since this organism is now grown on a large scale for incorporation into human and animal foodstuffs (Chaps. 25 and 26).

1.2 Past and Present

1.2.1 The Geological Record

The cyanobacterial record extends back to ~3,500 million years ago (Chap. 2). The considerable geological evidence for this comes from various sources, including microbially laminated structures known as stromatolites, cyanobacterial and cyanobacterium-like microscopic fossils, carbon isotopic data consistent with Rubisco-mediated CO_2-fixation being present and molecular data. It has proved important to have evidence from different sources, because of doubts raised as to whether the oldest structures were in fact biological. Chapter 2 also considers whether these organisms included O_2-producing photoautotrophic cyanobacteria much as known today, in spite of the fact that the Great Oxidation Event occurred a billion years later (~2,450 Ma ago). Whatever the sequence of changes may have been, they had a key role in the oxygenation of the atmosphere. Multicellularity in cyanobacteria is thought to have arisen between 2,450 and 2,220 Ma ago (Schirrmeister et al. 2011). Surprizingly, the extent to which cyanobacteria in later geological periods may have contributed to the petroleum deposits now being extracted seems much less clear (Chap. 16). It was concluded that in general the source, form, and distribution of oil-producing communities on the early Earth remains an enigma. However, there is considerable evidence for the involvement of free-living cyanobacteria and their symbiotic association with *Azolla* at particular sites with rich oil deposits.

Several authors have applied molecular data to assess the dates when modern cyanobacterial genera originated (e.g. Domínguez-Escobar et al. 2011). Although there is scope for discussion about the detailed conclusions, this approach should help geologists to consider their data more thoroughly, especially when morphologically complex cyanobacteria are present in geological samples. Such fossil materials are not only easier to equate with modern form-genera, but provide information on the environment where they were likely to have been growing, as is the case for *Palaeocalothrix* from the Precambrian described by Zhao-Liang (1984) (Chap. 22). In the case of hot and cold desert cyanobacteria, Bahl et al. (2011) concluded that the present-day distribution of taxa in the more extreme environments is determined by their ancient origins.

1.2.2 The Molecular Record

Until recently the majority of molecular studies on cyanobacteria focussed on taxonomic questions and phylogenetic relationships (Chaps. 7 and 22). Initially these were based on sequences of one particular gene or part of the genome, but studies began increasingly to combine information from several genes. When we introduced the previous "Ecology of Cyanobacteria" (Whitton and Potts 2000), there were complete sequence data for only a few cyanobacterial genomes. Now, such data are starting to be obtained more rapidly. Approximately 35 genomes were available with annotations for some 117,435 genes when Nakao et al. summarized the situation in 2010 and there were about 44 at the time of completing this chapter. Even where whole genome data are not available for a particular strain, genome sequences from other organisms can help to identify genes that interfere with phylogenetic reconstruction (Kauff and Büdel 2011). Extensive or complete genome sequences are driving significant advances in the understanding of not only phylogenetic relationships, but also the growth of populations *in situ* and in culture.

Phylogenetic analyses of 16S rDNA sequences led Schirrmeister et al. (2011) to suggest that all extant cyanobacteria may share a common ancestor, which was unicellular. Phylogenetic trees based on molecular data indicate that *Gloeobacter violaceus*, where the light-harvesting mechanism is restricted to the outer membrane of the cell rather than internal thylakoids, is the nearest living organism to that ancestor (see Kauff and Büdel 2011). However, Schirrmeister et al. also suggest that the majority of extant cyanobacteria descend from multicellular ancestors and that reversals to unicellularity have occurred at least five times. Among modern unicellular forms which have apparently originated from multicellular ones are the important marine *Synechococcus* and *Prochlorococcus* (Chaps. 5 and 20). Such concepts are a stimulus to research, but a great deal more evaluation of genomic information is needed before there is likely to be firm agreement about the detailed steps in cyanobacterial evolution. The fact that extant multicellular forms have similar, if not identical, morphologies to forms identified in early fossil records has sometimes surprized authors. However, if environmental factors influencing a past microbial community were closely similar to those of a modern one, there may have been little need for evolutionary change. The molecular evidence suggesting particular evolutionary steps needs to be compared with detailed environmental information for a particular geological time. This presents a huge challenge.

The consideration of multicellularity raises the question of the time of acquisition of heterocyst differentiation. Based on molecular data and theoretical modelling Rossetti et al. (2010) inferred, perhaps not unexpectedly, that terminally differentiated cyanobacteria evolved after undifferentiated species. The compartmentalization afforded by multicellularity is required to maintain the vegetative/heterocyst division. It is generally concluded that the heterocyst evolved only once because of the large number of steps involved (Henson et al. 2004), but the possibility of some dedifferentiation at various times should be borne in mind. There is a great deal of variation in heterocyst morphology and probably also functioning, which has as yet scarcely been investigated.

Based on a comparison of 58 contemporary cyanobacterial genomes, Larsson et al. (2011) concluded that the most recent common ancestor of cyanobacteria had a genome size of approx. 4.5 Mbp and 1,678–3,291 protein-coding genes, 4–6% of which are unique to cyanobacteria today. They concluded that there have been two routes of genome development during the history of cyanobacteria. One was an expansion strategy driven by gene-family enlargement which provides a broad adaptive potential. The other was a genome streamlining strategy which imposes adaptations to highly specific niches.

An important question absorbing the energy of many research groups is how a particular cluster or clusters of genes on a genome equates with particular phenotypes. The study by Larsson et al. (2011) led them to conclude that a few orthologues can be correlated with specific phenotypes, such as filament formation and symbiotic competence. Where organisms have diverged from each other relatively recently, they may be expected to have equivalent sets of genes in the same relative position on the genome. Recognition of this helps researchers to infer how portions of genomes are excised and transferred during the course of evolution. This approach (synteny) has provided useful insight for cyanobacteria. For instance, Stucken et al. (2010) compared the genomes with the smallest size of any filamentous species sequenced: *Cylindrospermopsis raciborskii* CS-505 i (3.9 Mbp) and *Raphidiopsis brookii* D9 (3.2 Mbp). Despite differences in their phenotypic features, these strains form a monophyletic group. The authors commented on the remarkable conservation in gene order between these genomes; differences in repetitive element content account for most of the difference in the genome. It was concluded that the lack of heterocysts in strain D9 is a secondary loss.

Local niche occupancy of any particular marine *Synechococcus* lineage appears to be driven by lateral gene transfer, in which specific genomic loci (islands) play a key role as a repository for transferred genes (Dufresne et al. 2008). This poses the question as to how important is the physical location of these islands. In a study with *Prochlorococcus* Kettler et al. (2007) asked whether flexible genes (as opposed to core genes) are located preferentially in island regions, and, if so, whether the most recently acquired genes are more likely to be island genes: are recently acquired genes directed to specific genomic loci? The authors identified genes that

appear to define high-light and low-light adapted phenotypes, but they also provided a detailed discussion and further questions relevant to cyanobacterial distribution and selection. "How many *Prochlorococcus* genotypes truly exist in the ocean, and what fraction of these has differential fitness at any point in time?" Information which Kettler et al. thought would be particularly enlightening is to understand the complete genome diversity of the 10^5 cells in a millilitre of ocean water, and, conversely, how widely separated in space two cells with identical genomes might be.

It would be hard to overstate the significance and complexity of these deliberations. For example, in the oligotrophic open ocean *Prochlorococcus* accounts for around half of all photosynthesis (Chaps. 5 and 20). Yet, remarkably, *Prochlorococcus* genomes lack catalase and other protective mechanisms that would appear essential for competition in the illuminated euphotic zone where reactive oxygen species are generated. It seems that genomic streamlining of *Prochlorococcus* through evolution was coincident with reliance on hydrogen peroxide-consuming members of the euphotic community (Morris et al. 2011). This emphasizes the importance of indirect biotic interactions in establishing niche boundaries and presumably driving genome evolution. Complex issues indeed, and it remains to be seen what further surprizes there are as studies continue on correlations between genomic form and function in cyanobacteria.

In addition to the examples already mentioned, molecular information has proved especially useful in a number of studies reported in this volume. This applies, for instance, to the study of the biosynthesis of microsporines, one of the types of molecule providing UV protection for cyanobacteria (Chap. 19). Balskus and Walsh (2010) used genome sequence data from different cyanobacteria in a genetic and molecular analysis of biosynthesis in *Anabaena variabilis* ATCC 29413. The mode of recruitment (reaction mechanism) of ATP-dependent peptide bond forming enzymes involved in this synthesis is apparently unprecedented in natural product biosynthesis. Of particular note is that the biosynthetic pathway is short, requiring only four enzymes. This is especially noteworthy in view of the ecological importance of tolerance to UV radiation.

1.3 Ecology: Current Challenges

1.3.1 Sensing the Environment and Other Organisms

An ability to detect and respond to variations in the environment is of key importance for the success of cyanobacteria on this planet and an understanding of which parts of the genome of a species are involved should prove a great stimulus for research. The following are aspects which seem of particular interest. Until recently most studies were concerned with light, N and P, but increasingly responses to the presence of other cells, whether of the same or a different species have gained attention. In the case of light, Castenholz (1983) concluded that most responses occur only after apparently random movements result in the long axis lying parallel to the light field. However, a range of photoreceptors are now known to exist in cyanobacteria for sensing light, such as the structure with carotenoid globules and rhodopsin-like pigment in the tip of the apical cell of a red-coloured *Leptolyngby*a (Albertano et al. 2000; Sect. 11.4.3). The first convincing evidence for chemotaxis came from Waterbury et al. (1985) for marine phycoerythrin-containing *Synechococcus* isolates showing swimming motility. The swimming behaviour, which was confined to open ocean isolates, showed a marked chemotactic response to various nitrogenous compounds (Willey and Waterbury 1989). The threshold levels for chemotactic responses were in the range 10^{-9}–10^{-10} M, which could be ecologically significant in the ocean. More recently the study of chemotaxis in cyanobacteria has focussed largely on the attraction of hormogonia to potential symbiotic partners. The first full account was for *Nostoc* and the liverwort *Blasia* (Knight and Adams 1996), but the phenomenon has now been shown for a range of associations (Nilsson et al. 2006; Chap. 23).

The study by Albertano et al. (2000) on the apical cell of a *Leptolyngbya* raises the question of the role of apical cells in this family, especially in species of *Phormidium*. At least a few trichomes of most Oscillatoriaceae populations have an apical cell which is in some way modified, such as being markedly pointed or with a decrease in colour. More distinct forms are a thickening at the end, a cap or an even more elaborate structure, the calyptra. It is unclear whether the thickening, cap and calyptra are distinct or there is a continuum between them, or whether they have different functions, but they are important characters used to distinguish species. Apart from this, there is a striking lack of detailed quantitative information on these structures, in spite of the fact that this could have been obtained any time in the past century. Although taxonomic accounts seldom make this clear, the specialized cell is present at only one end of a trichome. The frequency of trichomes with a modified end cell varies markedly between samples, so is presumably influenced by the environment. Should no trichome in a population possess a calyptra, it is impossible to comment on whether this is a genetic feature or merely a response to the environment. For the calyptra in particular, most field samples show only a few trichomes with this structure, so it is essential to check at least 20 trichomes to identify a sample.

The calyptra is probably the best known morphological structure in cyanobacteria about which nothing is known of its role. Perhaps the calyptra is involved in sensing light, as in the tip of the end cell of the cave *Leptolyngbya* studied by

Albertano et al. (2000), but it seems more likely that it is involved with another factor. We suggest that the most likely are phosphate gradients, the presence of other trichomes or possibly a combination of both. It is hard to understand how *Phormidium* mats with motile trichomes develop on submerged surfaces unless sensing between trichomes occurs. It is even more evident that sensing must occur between Rivulariaceae trichomes aggregating to form a colony (Chap. 22), though in this case the ends of the cells aggregating together are starting to differentiate a heterocyst. Is the heterocyst in this case involved in sensing other cells in addition to fixing N_2?

There have been many suggestions that quorum sensing is likely in be involved in cyanobacterial processes (e.g. Mann 2000) and some of the above must surely provide examples. Production of the toxin microcystin by *Microcystis* also seemed a likely example, so it was a surprize when several studies failed to find evidence for factors such as cell density changes influencing transcription of the microcystin gene cluster (Dittmann et al. 2001; Braun and Bachofen 2004; Pearson et al. 2004). ^{14}C studies with *M. aeruginosa* PCC 7806 showed that when an intracellular pool of microcystin was built up there was no significant export from the cells and the authors (Rohrlack and Hyenstrand 2007) interpreted this as a lack of evidence for quorum sensing. However, Chap. 7 shows how *Microcystis* responds in various ways to the presence of grazers, including changes in colony morphology and microcystin content. *Microcystis* strains exposed to zooplankton increased their cell specific toxin production (Jang et al. 2007; Sect. 7.5.1). Other aspects of signalling are discussed in Chap. 18, such as possible links between cyanobacterial-derived extracellular signalling molecules and phage physiology (Sect. 18.3) and autoinduction systems (Sect. 18.4). Evidence for the importance of quorum sensing in the regulation of surface phosphomonoesterase activity by epibiontic bacteria associated with *Trichodesmium* colonies was shown by Van Mooy et al. (2012), but it not yet clear how this influences P acquisition by *Trichodesmium* itself.

Microcystis is not the only cyanobacterium known to show morphological changes in response to the presence of grazers. Fialkowska and Pajdak-Stós (1997) found that when two *Phormidium* isolates from very shallow pools were subjected to grazing pressure by the ciliate *Pseudomicrothorax dubius*, both strains showed significant increases in the number of filaments terminating in an empty sheath. There was active withdrawal of a trichome inside a sheath when disturbed by grazers. *P. dubius* was unable to ingest trichomes enclosed in a sheath. *Phormidium* may be less efficient under these conditions, perhaps because of reduced nutrient uptake. However, possession of a sheath was also likely to have been important for these populations which occurred in an environment likely to become dried out intermittently. Another example of a response to a grazer is that of microcolony formation by a strain of *Cyanobium* sp. from single cells; this was induced by the presence of the photophagotroph, *Ochromonas* sp. DS (Jezberová and Komárková 2007). Colonies were characterized by hundreds of tubules (spinae), 100 nm to 1 μm long and 63 ± 6 nm wide on the surface of *Cyanobium* cells cultured together with *Ochromonas*. Such spinae have been reported a number of times on single-celled cyanobacteria, so perhaps this is a widespread response. In any case it seems probable that there are numerous other examples of cyanobacterial morphological responses to grazers waiting to be discovered. Perhaps because of their larger size, there is considerably more known about induced morphological and chemical responses of eukaryotic algae than cyanobacteria (see Van Donk et al. 2011).

1.3.2 Nitrogen and Phosphorus

While P has long been identified as the most common limiting nutrient in freshwater ecosystems (e.g. Schindler 1977), earlier studies focussed on uptake of P_i, because of the increased concentrations in lakes and rivers due to human activity and the resulting problems of cyanobacterial blooms. When considering how cyanobacteria and eukaryotic algae acquire P efficiently if the element is in short supply, some authors (e.g. Wagner and Falkner 2001) have considered only P_i, but others review all possibilities (Dignum et al. 2005). This matters, because, away from human activity, it seems likely that P acquisition from organic sources is more important than Pi for most cyanobacteria. It is difficult to be sure of the situation, because there are few really detailed studies on the P fractions present in freshwater and it is doubtful if any freshwater sample has ever been studied sufficiently to characterize all the P-containing molecules reaching concentrations of possible use for a cyanobacterium. There have been no studies on the possible presence and utilization of phosphonates in freshwater, in spite of their known importance for several marine cyanobacteria, such as *Trichodesmium* (Dyhrman et al. 2006). However, most filamentous cyanobacteria can obtain P from a wide range of organic molecules, though not necessarily all, and there are differences between species (Whitton et al. 1991, 2005). The overall situation with unicellular cyanobacteria is less clear, because of doubts about the relevance of data obtained with strains cultured with P_i for many generations. However, many strains can use phosphomonoesters, including the marine *Crocosphaera watsonii* (Dyhrman and Haley 2006). The evidence suggests that unicellular forms may be less successful at using phosphodiesters (Whitton et al. 1991). The next research step should be to relate the ability of particular strains to acquire different forms of P to the types and concentrations of the various molecules

in their natural environment. Inorganic precipitates and inorganic – organic complexes, such as the brown deposits among the mucilage of some planktonic colonial Chroococcales (e.g. *Cyanogranis ferruginea*), are likely to include P of potential use to the organism.

Although the problems associated with P concentrations in culture collection media different from the concentrations in the natural environment are a particular worry when considering unicellular strains, phenotypic and probably also genetic changes can also occur during prolonged subculture of filamentous species (Chap. 22). The difficulties originated from the fact that culture media mostly used a phosphate buffering system until the mid-1970s. For instance, the medium of Kratz and Myers (1955), which was often used for cyanobacteria over the next 20 years, has 158 mg L^{-1} PO_4-P. This needs to be borne in mind when reading research results obtained during this period, especially those concerning N_2 fixation. Although organic buffers started to be introduced in the 1970s, many media still had P concentrations well in excess of those likely in nature. BG-11 medium (Allen and Stanier 1968; Rippka et al. 1979) has been the most widely used medium for cyanobacteria, but sometimes without combined N ($BG11_0$). Both versions have 5 mg L^{-1} P, although more recently the concentration has sometimes been reduced to reduced 1 or 2 mg L^{-1} P. Even the well-known Chu No.10 medium (Chu 1942), which was designed for growing lake algae in the laboratory, has 2.87 mg L^{-1} P. All these and most other media listed by Andersen et al. (2005) still have P concentrations far higher than typical in nature. The N concentrations are usually also high, but below the value for the N:P ratio likely to lead to P limitation (16:1 molar, 7.2:1 by mass: Redfield et al. 1963), even if, in the case of a batch culture, a sufficiently high biomass is reached for this to occur.

1.4 Taxonomy and Nomenclature

It may seem a statement of the obvious to comment that different people have different reasons for wanting to name a cyanobacterium. However, cyanobacteria have sometimes had a reputation for being difficult to name and part of the reason for this comes from the fact that several different taxonomic approaches have been introduced at various times and none are ideal for all the needs of people requiring names. It is difficult to understand the present situation without knowing something of the past. The following account is mainly for those who know little about the subject; several reviews published in recent years provide much more detailed information.

Staff in water companies with a single reservoir, who will probably know the organism as a blue-green alga in an English-speaking country, merely want to give the same name to the same organism each time it is encountered. Larger environmental organizations require a more rigorous set of names which is consistent within their area, while ecologists conducting field surveys aim to use names which are consistent world-wide. Information about cyanobacterial populations in many of the world's ecosystems is still very limited and the need to provide detailed floristic lists for ecological studies is likely to increase greatly. Most of the generic and many of the species names used for this purpose originated in the second half of the nineteenth century and the first half of the twentieth century – what may be considered classical taxonomy. However, the names are increasingly being modified by results from molecular studies. Many of the problems for non-specialists are similar to those discussed with clarity and sympathy by Stace (2010) for field botanists wanting to name angiosperms, but who are not professional taxonomists.

Molecular data have provided a great stimulus not only for the broader questions of cyanobacterial evolution and phylogenetics discussed in Sect. 1.2.2, but also more straightforward matters of cyanobacterial taxonomy. They have helped to distinguish organisms which most phenotypic characters suggest are quite similar, a particular problem with many unicells; *Microcystis* provides an example (Chap. 7). Molecular data have also ensured the speedy acceptance of splits in well-known genera, where obvious morphological differences had been ignored in the past, as has occurred in the separation of *Cuspidothrix* (Rajeniemi et al. 2005) and *Sphaerospermum* (Zapomělová et al. 2009) from *Aphanizomenon.* However, molecular data have not always been used with sufficient care in phylogenetic and taxonomic studies. This is sometimes merely because of the rush to publish, but other times it comes from a failure to appreciate the significance of how taxa were originally described.

Many characters used in the original descriptions were ones which the organism had evolved to respond to what we might now consider as "stress" factors, although in most cases the original authors had no or little idea about such environmental factors. These include the various types of sheath pattern in terrestrial forms, many of which are responses to different cycles of water availability and the formation of sheath pigments to protect from UV damage. The influence of combined N on heterocyst formation by many cyanobacteria became increasingly clear during the 1970s (Wolk 1983), but recognition of the importance of P in inhibiting the formation of multicellular hairs in cyanobacteria has been less widely recognized (Chap. 22). Nevertheless, about 16% of the filamentous species listed by Geitler (1932) form such hairs.

Akinetes or other resting stages are needed to identify individual species in genera such as *Anabaena* and *Cylindrospermum*, so again these will only be seen if the correct environment is provided. It is essential to name an

organism when first isolated from nature if the name is to be used in phylogenetic analysis. Giving the correct name to a culture is even more of a challenge if there is a need to consider the whole population. This is essential, for instance, in Rivulariaceae colonies, which may contain more than one genotype (Berrendero et al. 2008), in *Microcystis* (Chap. 7) and probably also in all *Phormidium* (see above). Perhaps the hardest of all is to give the right name when the morphology of a cyanobacterium responds to the presence of a grazer.

The classical taxonomic information was first consolidated by Geitler (1932) and the approach to naming blue-green algae continued much the same for the next 40 or so years, although with a lot more information being incorporated, some of it based on experimental studies during the latter part of this period. The guidelines for naming organisms were provided by the International Code of Botanical Nomenclature (ICBN). In a series of monographs Francis Drouet set out to replace the pragmatic approach of the classical system with one based on reducing the number of genera to one relying solely on the most obvious characters (e.g. Drouet 1968), eventually reducing the number to nine (Drouet 1981). The monographs are an excellent source of nomenclatural information, but have little practical value for naming organisms. However, Drouet's views led to heated discussion at many symposia up to as late as 1991. There would be little need to mention them now but for the fact that an earlier version of Drouet's names was used for the colour pictures shown by Palmer (1962), which were subsequently reproduced in many editions of *Standard Methods for Water and Wastewater Treatment* published by the American Public Health Association. These pictures have probably been seen by more people than any others of cyanobacteria and are still on the walls of many water treatment laboratories around the world.

A third approach is that introduced by R.Y.Stanier, who became convinced during the mid-1970s that the classical approach was inadequate for critical research. A meeting of blue-green algal specialists – authors of floras and monographs – at Kastanienbaum by the Zürichsee in Switzerland gave him the chance to ask them to use light microscopy to name the genera of 20 cultures from the Pasteur Culture Collection. (B.A.W. was one of those involved.) Comparisons of the lists obtained showed that many organisms had been given more than one name and, in the case of several filamentous forms, *Oscillatoria*, *Lyngbya* and *Phormidium* for the same material. This added weight to the argument that organisms should be treated like bacteria, with isolation of individual cells or filaments to permit measurements of a wide range of characters. He therefore proposed that their taxonomy should follow the rules of International Code of Nomenclature of Bacteria, together with a change in name to cyanobacteria (Stanier et al. 1978); the practical methods were described by Rippka et al. (1979). Two editions of *Bergey's Manual of Determinative Bacteriology* have provided accounts of the genera which the authors of the cyanobacterial chapters thought could be characterized clearly at the time. The first (Castenholz and Waterbury 1989) relies largely on phenotypic characters, whereas the second (Castenholz 2001) makes considerable use of molecular information, especially sequence data.

The 1978 proposal by Stanier et al. led to nomenclatural problems which are still not fully resolved. The earlier steps towards doing this were reviewed by Oren (2004), who stressed the need for botanical and bacteriological taxonomists to use unified rules to describe new taxa. Oren (2011) assessed the contents of all the papers on cyanobacterial systematics and nomenclature published in the *International Journal of Systematic Bacteriology* and the *International Journal of Systematic and Evolutionary Microbiology* (and a predecessor bulletin). There have been only very few descriptions of new cyanobacterial taxa under the rules of the International Code of Nomenclature of Prokaryotes (ICNP) because of the difficulty of validly publishing new names of cyanobacteria under its rules. Most descriptions of new taxa are still published in the botanical literature. The situation had not changed much since Oren and Tindall (2005) considered how successful the system was in which Cyanophyta/Cyanobacteria can be named according to the provisions of either code. The problems include the fact that valid publication under the ICNP rules requires publication in a particular journal, whereas that of the ICBN has no such restriction. Another difference is that the ICNP requires the nomenclatural type of a species to be a viable type strain maintained in pure culture, while under the ICBN, non-living type specimens must be preserved permanently, although algal cultures preserved in a metabolically inactive state are acceptable as types. Neither system deals effectively with the problem of genetic shifts in cultures, which may even have occurred by the time the material is designated a type culture, nor the fact that they may only express characteristic features when present as a population.

At the same time as the bacteriological approach has been developing, there have been many reports of new taxa and nomenclatural revisions based on the ICBN rules, which have themselves been changing. This literature was brought together for the Chroococcales by Komárek and Anagnostidis (1999) and by the same authors for the Oscillatoriales in 2005. Often the previous revisions had also been made by them; some of these were made with more evidence to support them than others. The two volumes assemble a wealth of information and are essential for anyone making broad surveys. However, it would be a challenge to make such a survey without practical advice from others with experience of how their system is put into practice. There are, for instance, 109 species of *Phormidium* listed and the authors state that

200 species have been recognized and are identifiable. However, most morphological characters are influenced by the environment and often merge into those of other species.

Nomenclatural revisions in the past 10 years almost all incorporate molecular data and in many cases this is what led to the decision to make the change. Hoffmann et al. (2005) stressed that the taxonomic system needed to be continually revized and updated, but that system is essentially a continuation of the traditional system. Komárek (2010) reviewed the situation and emphasized the need for molecular data to have a central role, but also that phenotypic and ecological characters must be an integral part of the generic definition. He indicated that the molecular definition of a gene sequence corresponding to the genus should be based on a similarity index of ±95% using 16S rRNA sequencing. The species concept is "not uniform and must be modernized according to the diverse nature of genera". He regretted that "molecular cyanobacteriologists pay attention to the use of molecular methods for taxonomic articles, but unfortunately do not accept the results of modern investigations into cyanobacterial diversity in their studies and strain collections".

It seems probable that progress will continue much as indicated by Hoffmann et al. (2005), with a continual update of what originated from the classical system, but with an ever increasing contribution from molecular data. However, the situation with unicellular forms (in the broadest sense) is rather different from that of filamentous ones, where there are often quite a number of morphological characters. It might have been better if Stanier et al. (1978) had restricted their suggestion about change in nomenclatural code 1978 to the Chroococcales, with consideration of the filamentous forms, which are the main part of floristic surveys, being left until later. It will probably always be essential to rely on molecular data for reliable identification of many unicellular forms, but there is still a lot of potential for improving the traditional system enough to make it is possible to allocate a meaningful binomial name to the majority of filamentous forms found in nature based on their morphology.

Proposals to revize generic limits should be deferred until there are data for a sufficient number of strains. We believe that many nomenclatural changes have been introduced much too rapidly and this will inevitably lead to further revisions in a few years time. It is for this reason that not all recent nomenclatural changes have been included in the revized floristic account of cyanobacteria in the British Isles (Whitton 2011). However, in the long-term by far the most effective way to deal with the practical need for names in detailed field surveys is to use an interactive identification system based on morphology, although molecular comparisons should be sufficient for rapid checks on potential problem organisms. A early attempt at developing an interactive system was provided by Whitton et al. (2003) using the Lucid software prepared by University of Brisbane, Australia, and with the information on a CD. It should now be possible to provide a system which permits the storage of taxonomic information and records from as many countries as there are data, rapid conversion between different nomenclatural conventions and synonyms, a large number of images and rapid access to the internet. The information could also be linked to molecular records.

The increasing need to include sequence comparisons with the GenBank database in taxonomic comparisons and phylogenetic studies makes it is essential for the names to be correct. As pointed out by Komárek (2010), this is not always so, and some of the reasons for this have been explained above. It would be useful to have an index of reliability for every name in the database, although this would require retrospective assessment for the names already there. There is also a need for ecological relevance to be among the criteria used to decide which strains are used for complete genome sequencing. This should include detailed information about its original environment and morphology and the use of material which has had minimal chance for genetic change since first isolated.

Finally, we would point out that detailed descriptions of the morphology and cell contents visible with a light microscope of populations of filamentous forms at a field site can tell a lot about the environment at that site without even knowing the name of the organism. This comes from an understanding of the factors leading to the "stress" characters mentioned above and others details such as the relative abundance of cyanophycin (N storage) and polyphosphate (P storage) granules. The more morphologically complex the organism, the more information can be deduced.

1.5 The Future: How Cyanobacteria Can Contribute to Solving Real Problems

The variety of ways in which cyanobacteria are currently used for practical purposes is likely to surprize many readers of Chap. 26. Some of the places where cyanobacteria are harvested locally are well known, like the surrounds of Lake Chad, but others much less so, such as in Myanmar. It seems likely that there is a lot more to be reported about local use of natural material, especially from S-E. Asia. The commercial cultivation of *Arthrospira* ("Spirulina") continues to increase and what may have seemed at times rather wild suggestions for production have frequently turned into reality. Those of us who spent an afternoon at the 2005 Applied Phycology Symposium in Kunming listening to the plans of Chinese staff for managing large-scale cultivation on the Ordos Plateau in Inner Mongolia can now read in Chap. 25 about current production. Hopefully the comment by Lu et al. (2011) that a plan for a future annual production of 10^6 that has been sketched out will also become a reality.

Another success from the dry parts of China is the use of cyanobacterial inocula in the improvement of soils in semi-desert regions (Chap. 12). This has shown how important it is to study the ecology of natural soil biological crusts, select strains adapted to a particular area and then to find out how the material should be grown and applied to sites in that area. The sequencing of the genome of a strain of *Microcoleus vaginatus* (Starkenburg et al. 2011), one of the main species used in inocula, should assist in optimizing strains for a particular region.

There is a long history of cyanobacteria being applied to fields in other regions to increase soil fertility, especially the N status of rice fields. There has been considerable success in the use of *Azolla*, with its N_2-fixing symbiont, but most of the earlier studies on free-living cyanobacteria were too fragmentary to have much practical success. In particular there was often a failure to obtain an understanding of the ecology of local sites (Whitton 2000). However, some successes have been reported in recent years (Sect. 26.5.5). Although rice fields are much more complex ecosystems than soil biological crusts, it should be possible to manage the cyanobacterial populations of rice fields to enhance soil fertility effectively once the same critical and long-term approach is applied as has been done for semi-desert soils in China. The problems associated with cyanobacterial damage to outdoor monuments and archaeologically important surfaces in caves and other underground sites provide another example where detailed ecological research has helped to provide solutions (Chap. 11). Dealing with problems without such ecological understanding has sometimes done more harm than good and is still continuing to do so at many sites, especially large outdoor monuments in south, south-east and east Asia.

The use of barley (*Hordeum vulgare*) straw to control cyanobacterial blooms provides an example of an ecological problem where there have been many studies, but none adequate enough to ensure that the solution is always effective. The studies are summarized by Ó hUallacháin and Fenton (2010). Release of polyphenolics from rotting stems has been suggested to be the main factor involved (Pillinger et al. 1994; Everall and Lees 1997) and there is evidence for 1,000–3,000 molecular weight range polyphenolics being toxic to *Microcystis aeruginosa* (Waybright et al. 2009). However, evidence for other factors such as increases in zooplankton grazer density and microbial activity has been found for particular sites. In addition it is possible rotting barley straw might release other toxic molecules harmful to cyanobacterial blooms, since Wu et al. (2011) showed that periphyton biofilms could produce water-soluble allelochemicals such as indole and 3-oxo-a-ionone, which led to marked inhibition of cyanobacterial growth. Marked differences have been found in the responses of different planktonic cyanobacteria and eukaryotic algae (Brownlee et al. 2003).

Barley straw is now used widely in the British Isles to control cyanobacterial blooms, and, to a lesser extent, eukaryotic algae. It is also in increasing use elsewhere, though more often in ornamental ponds than reservoirs. Tests in North America have led to only mixed success (Boylan and Morris 2003; Geiger et al. 2005), perhaps due to different barley cultivars or higher rates of N fertilization in the barley fields reducing the lignin content of the straw. Nevertheless there is convincing evidence for success in shallow, well aerated waters in the British Isles, when a sufficient density of bales of straw is applied early enough for rotting to be well underway by the time a bloom population would normally start to increase – typically late spring. The value of straw used for this purpose is sufficient to influence borderline decisions by some farmers about the area to be planted for barley. There is great potential for making the barley straw methodology much more effective, but anyone planning a research project would be well advized to read the critical comments of Ó hUallacháin and Fenton (2010) on the weaknesses of previous studies.

In view of the widespread occurrence of cyanobacterial blooms in tropical and subtropical waters used for drinking water, the possibility that the straw of some rice cultivars might be used in a similar way should be tested. Evidence in support of this comes from an experimental study by Rice et al. (1980) showing that decaying rice straw had an inhibitory effect on cyanobacterial growth and N_2 fixation. In addition, anecdotal reports from deepwater rice farmers in Bangladesh to B.A.W. indicate that leaving rice straw to rot on soils after harvest at the end of the flood period decreases winter growths of cyanobacteria on the soil surface.

The intense interest in the potential of cyanobacteria for various products is leading to an exploration of the ways of optimizing the cell physiology of strains if it is to be used to produce the product, or the isolation and transfer of important cyanobacterial operons to non-phototrophic organisms if these are cheaper to grow on an industrial scale. Biofuel (Chaps. 16 and 26) is of course the most important product needed. Significantly, an alkane biosynthesis pathway from cyanobacteria as diverse as *Cyanothece* and *Nostoc* spp. is described recently by Schirmer et al. (2010). This pathway consists of an acyl–acyl carrier protein reductase and an aldehyde decarbonylase that together convert intermediates of fatty acid metabolism to alkanes and alkenes. Heterologous expression of the cyanobacterial alkane operon in *Escherichia coli* led to the production and secretion of long-chain alkanes and alkenes. Another approach is genetic modification of strains such as *Synechocystis* PCC 6803 wild type (SD100) to produce and secrete fatty acids (Liu et al. 2011). Since this involves changes to the cell walls, and cell density dependent changes may damage cell membranes, it remains to be seen how well such strains succeed, perhaps in competition with others, under rigorous environmental conditions.

Assessment of whole genome sequences would seem to be the most logical approach for long-term plans for genetic modification. The study carried out by Jones et al. (2011) on *Lyngbya majuscula* 3L provides an example. This strain belongs to a pantropical species and a worldwide genus that is the source of some 35% of all reported cyanobacterial natural products. In spite of the fact that some *L. majuscula* strains fix N_2 (Lundgren et al. 2003), no evidence for nitrogenase genes was found in *L. majuscula* 3L. However, this strain does produce curacin A, a tubulin polymerization inhibitor, and the molluscicide barbamide. Jones et al. suggested that *Lyngbya* metabolites are strain-specific and may be useful in delineating species. They showed that this species has a complex gene regulatory network with a large number of sigma factors and other regulatory proteins, it was concluded that this shows an enhanced ability for environmental adaptation or for forming microbial associations. More such detailed analyses of genome sequence are needed to provide a rational basis for assessing how strains interact with their environment and which ones are most likely to form products of potential biotechnological use.

References

Albertano P, Barsanti L, Passarelli V, Gaultieri P (2000) A complex photoreceptive structure in the cyanobacterium *Leptolyngbya* sp. Micron 31:27–34

Allen MM, Stanier RY (1968) Growth and division of some unicellular blue-green algae. J Gen Microbiol 51:199–202

Andersen RA, Berges JA, Harrison PJ, Watanabe MM (2005) Appendix A – Recipes for freshwater and seawater media. In: Andersen RA (ed) Algal culturing techniques. Elsevier Academic Press, Amsterdam, pp 429–532, 578 pp

Bahl J, Lau MCY, Smith GJD, Vijaykrishna D, Cary SC, Lacap DC, Lee CK, Papke RT, Warren-Rhodes KA, Wong FKY, McKay CP, Pointing SB (2011) Ancient origins determine global biogeography of hot and cold desert cyanobacteria. Nat Commun 2:163

Balskus EP, Walsh CT (2010) The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. Science 329:1653–1656

Berrendero E, Perona E, Mateo P (2008) Genetic and morphological characterization of *Rivularia* and *Calothrix* (Nostocales, Cyanobacteria) from running water. Int J Syst Evol Microbiol 58:447–454

Bothe H, Tripp HJ, Zehr JP (2011) Unicellular cyanobacteria with a new mode of life: the lack of photosynthetic oxygen evolution allows nitrogen fixation to proceed. Arch Microbiol 192(10): 783–790

Boylan JD, Morris JE (2003) Limited effects of barley straw on algae and zooplankton in a midwestern pond. Lake Reserv Manage 19:265–271

Braun W, Bachofen R (2004) Homoserine-lactones and microcystin in cyanobacterial assemblages in Swiss lakes. Hydrobiologia 522:271–280

Brownlee EF, Sellner SG, Sellner KG (2003) Effects of barley straw (*Hordeum vulgare*) on freshwater and brackish phytoplankton and cyanobacteria. J Appl Phycol 15:525–531

Castenholz RW (1983) Motility and taxes. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 413–440, 688 pp

Castenholz RW (2001) Phylum BX. Cyanobacteria. Oxygenic photosynthetic bacteria. In: Boone DR, Castenholz RW (eds) Bergey's manual of systematic bacteriology, 2nd edn. Springer, New York (with individual sections by different groups of authors)

Castenholz RW, Waterbury JB (1989) Cyanobacteria. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams & Wilkins, Baltimore, pp 1710–1727

Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB, Weischmeyer NA (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature 334:340–343

Chu SP (1942) The influence of the mineral composition of the medium on the growth of planktonic algae. Part I. Methods and culture media. J Ecol 30:284–325

Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407

Díez B, Bergman B, El-Shehawy R (2008) Marine diazotrophic cyanobacteria: out of the blue. Plant Biotechnol 25:221–225

Dignum M, Matthijs HCP, Pel R, Laanbroek HJ, Mur LR (2005) Nutrient limitation of freshwater cyanobacteria. In: Huisman J, Matthijs CP, Visser PM (eds) Harmful cyanobacteria. Springer, Dordrecht, pp 65–86, 241 pp

Dittmann E, Erhard M, Kaerbernick M, Scheler C, Neilan BA, von Dühren H, Borner T (2001) Altered expression of two-light dependent genes in a microcystin-lacking mutant of *Microcystis aeruginosa* PCC 7806. Microbiology 147:3113–3119

Domínguez-Escobar J, Beltrán Y, Bergman B, Díez B, Ininbergs K, Souza V, Falcón LI (2011) Phylogenetic and molecular clock inferences within *Rivulariaceae* from distant environments. FEMS Microb Lett 316(2):90–99

Drouet F (1968) Revision of the classification of the oscillatoriaceae, Monographs of the Academy of Natural Sciences, 15. Academy of Natural Sciences, Philadelphia, 334 pp

Drouet F (1981) Summary of the classification of blue-green algae. Beih Nova Hedwigia 66:135–209

Dufresne A, Ostrowski M, Scanlan DJ, Garczarek L, Mazard S, Palenik BP, Paulsen IT, Tandeau de Marsac N, Wincker P, Dossat C, Ferriera S, Johnson J, Post AF, Hess WR, Partensky F (2008) Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. Genome Biol 9(5):R91-16

Dyhrman ST, Haley ST (2006) Phosphorus scavenging in the unicellular marine diazotroph *Crocosphaera watsonii*. Appl Environ Microbiol 72:1452–1458

Dyhrman ST, Chappell PD, Haley ST, Moffett JW, Orchard ED, Waterbury JB, Webb E (2006) Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature 439:68–71

El-Shehawy R, Lugomela C, Ernst A, Bergman B (2003) Diurnal expression of *hetR* and diazocyte development in the filamentous non-heterocystous cyanobacterium *Trichodesmium erythraeum*. Microbiology 149:1139–1146

Everall NC, Lees DR (1997) The identification and significance of chemicals released from decomposing barley straw during reservoir algal control. Water Res 31:614–620

Fialkowska E, Pajdak-Stós A (1997) Inducible defence against a ciliate grazer, *Pseudomicrothorax dubius*, in two strains of *Phormidium* (cyanobacteria). Proc R Soc Lond B 264:937–941

Garcia-Pichel F, Belnap J, Neuer S, Schanz F (2003) Estimates of global cyanobacterial biomass and its distribution. Algol Stud 109:213–227

Garcia-Pichel F, Ramírez-Reinat E, Gao Q (2010) Microbial excavation of solid carbonates powered by P-type ATPase-mediated transcellular Ca^{++} transport. PNAS 107(50):21749–21754

Geiger S, Henry E, Hayes P, Haggard K (2005) Barley straw – algae control literature analysis. Available on internet in 2011 as barleyworld.org/barleystraw/Barley%20Straw%20-%20Algae%20Control%20Lit%20Anal%20Final.pdf

Geitler L (1932) Cyanophyceae. In: Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz 14. Akademische Verlagsgesellschaft, Leipzig, 1356 pp

Henson BJ, Hessbrock SM, Watson LE, Barnum SR (2004) Molecular phylogeny of the heterocystous cyanobacteria (subsections IV and V) based on *nif*D. Int J Syst Ecol Microbiol 54:493–497

Hoffmann L, Komárek J, Kaštovský J (2005) System of cyanoprokaryotes (cyanobacteria) – system in 2004. Algol Stud 117(Cyanobacterial Research 6):95–115

Jang M-H, Jung J-M, Takamura N (2007) Changes in microcystin production in cyanobacteria exposed to zooplankton at different population densities and infochemical concentrations. Limnol Oceanogr 52(4):1454–1466

Jezberová J, Komárková J (2007) Morphological transformation in a freshwater *Cyanobium* sp. induced by grazers. Environ Microbiol 9:1858–1862

Jones AC, Monroea EA, Podell S, Hess WR, Klages S, Esquenazia E, Niessene S, Hoover H, Rothmann M, Laskeng RS, Yates JR III, Richard Reinhardt R, Kube M, Burkart MD, Allen EE, Dorrestein PC, Gerwick WH, Gerwick L (2011) Genomic insights into the physiology and ecology of the marine filamentous cyanobacterium *Lyngbya majuscula*. Proc Natl Acad Sci USA 108:8815–8820

Kauff F, Büdel B (2011) Phylogeny in cyanobacteria: an overview. Prog Bot/Fortschr Bot 72:209–224

Kerbrat AS, Amzil Z, Pawlowiez GS, Sibat M, Darius HT, Chinain M, Laurent D (2011) First evidence of palytoxin and 42-hydroxy-palytoxin in the marine cyanobacterium *Trichodesmium*. Mar Drugs 9:543–560

Kettler G, Martiny AC, Huang K, Zucker J, Coleman ML, Rodrigue S, Chen F, Lapidus A, Ferriera S, Johnson J, Steglich C, Church G, Richardson P, Chisholm SW (2007) Patterns and implications of gene gain and loss in the evolution of *Prochlorococcus*. PLoS Genet 3:e231

Knight CD, Adams DG (1996) A method for studying chemotaxis in nitrogen-fixing cyanobacterium-plant symbioses. Physiol Mol Plant Pathol 49:73–77

Komárek J (2010) Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept). Hydrobiologia 639:245–259

Komárek J, Anagnostidis K (1999) Cyanoprokaryota 1. Teil. Chroococcales. In: Ettl H, Gärtner G, Heynig H, Mollenhauer D (eds) Süßwasserflora von Mitteleuropa 19/1. Gustav Fisher, Jena/Stuttgart/Lübeck/Ulm, 548 pp

Komárek J, Anagnostidis K (2005) Cyanoprokaryota: 2.Teil/Part 2. Oscillatoriales. In: Büdel B, Krienitz L, Gärtner G, Schagerl M (eds) Süßwasserflora von Mitteleuropa 19/2. Spektrum/Elsevier, Heidelberg, 759 pp (Reprinted in 2008 by Spektrum/Springer)

Koprowska L (1995) Sukcesja fauny dennej w zbiornikach powstałych po wydobyciu wegla brunatnego [Succession of bottom fauna in lignite post-mining reservoirs]. Olsztyn, 112 pp

Kosten S, Huszar VLM, Bécares E, Costa LS, Van Donk E, Hansson L-A, Jeppesen E, Kruk C, Lacerot G, Mazzeo N, DE Meester L, Moss B, Lürling M, Nöges T, Romo S, Scheffer M (2012) Warmer climates boost cyanobacterial dominance in shallow lakes. Glob Change Biol 18:118–126

Kratz WA, Myers J (1955) Nutrition and growth of several blue-green algae. Am J Bot 42:282–287

Larsson J, Nylander JAA, Bergman B (2011) Genome fluctuations in cyanobacteria reflect evolutionary, developmental and adaptive traits. BMC Evol Biol 11:187–208

Liu X, Sheng J, Curioss R (2011) Fatty acid production in genetically modified cyanobacteria. Proc Natl Acad Sci USA 108(17): 6899–6904

Lu Y-M, Xiang W-Z, Wen Y-H (2011) *Spirulina* (*Arthrospira*) industry in Inner Mongolia of China: current status and prospects. J Appl Phycol 23:264–269

Lundgren P, Lugomeia C, Söderback E, Bergman B (2003) Reevaluation of the nitrogen fixation behavior in the marine non-heterocystous cyanobacterium *Lyngbya majuscula*. J Phycol 39(2):310–314

Mann NH (2000) Detecting the environment. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 367–395, 669 pp

Morris JJ, Johnson ZI, Martin J, Szul MJ, Keller M, Zinser ER (2011) Dependence of the cyanobacterium *Prochlorococcus* on hydrogen peroxide scavenging microbes for growth at the ocean's surface. Plos One 6(2):1–13

Nakao M, Okamoto S, Kohara M, Fujishiro T, Fujisawa T, Sato S, Tabata S, Kaneko T, NakamuraY (2010) CyanoBase: the cyanobacteria genome database update 2010. Nucleic Acid Res 38 (Database issue):D379–D381. doi:10.1093/nar/gkp915

Nilsson M, Rasmussen U, Bergman B (2006) Cyanobacterial chemotaxis to extracts of host and nonhost plants. FEMS Microbiol Ecol 55:382–390

Ó hUallacháin D, Fenton O (2010) Barley (*Hordeum vulgare*)-inducated growth inhibitors of algae: a review. J Appl Phycol 22:651–658

Oren A (2004) A proposal for further integration of the cyanobacteria under the Bacteriological Code. Int J Syst Evol Microbiol 54:1895–1902

Oren A (2011) Cyanobacterial systematics and nomenclature as featured in the International Bulletin of Bacteriological Nomenclature and Taxonomy/International Journal of Systematic Bacteriology/International Journal of Systematic and Evolutionary Microbiology. Int J Syst Evol Microbiol 61:1–15

Oren A, Tindall BJ (2005) Nomenclature of the cyanophyta/ cyanobacteria/ cyanoprokaryotes under the International Code of Nomenclature of Prokaryotes. Algol Stud 117:39–52

Palmer CM (1962) Algae in water supplies. U.S. Department of Health, Education, and Welfare Division of Water Supply and Pollution Control, Washington, DC, 88 pp

Pearson LA, Hisbergues M, Börner T, Dittmann E, Neilan BA (2004) Inactivation of an ABC transporter gene, mcyH, results in loss of microcystin production in the cyanobacterium *Microcystis aeruginosa* PCC 7806. Appl Environ Microbiol 70:6370–6378

Pierce J, Omata T (1988) Uptake and utilization of inorganic carbon by cyanobacteria. Photosynth Res 16:141–154

Pillinger J, Cooper JA, Ridge I (1994) Role of phenolic compounds in the antialgal activity of barley straw. J Chem Ecol 20(7):1557–1569

Rajeniemi P, Komárek J, Hrouizek P, Willame R, Kaštovská K, Hoffmann L, Sivonen K (2005) Taxonomic consequences from the combined molecular and phenotype evaluation of selected *Anabaena* and *Aphanizomenon* strains. Algol Stud 117:371–376

Redfield AC, Ketchum BH, Richards FA (1963) The influence of organisms on the composition of sea water. In: Hill MH (ed) The sea, vol 2. Wiley-Interscience, Hoboken, pp 26–77

Rice EL, Lin C-Y, Huang C-Y (1980) Effects of decaying rice straw on growth and nitrogen fixation of a blue-green alga. Bot Bull Acad Sin 21:111–117

Rippka R, DeRuelles JB, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignment, strain histories, and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Rohrlack T, Hyenstrand P (2007) Fate of intracellular microcystins in the cyanobacterium *Microcystis aeruginosa* (Chroococcales, Cyanophyceae). Phycologia 46:277–283

Rosetti V, Schirrmeister BE, Bernasconi MV, Bagheri HC (2010) The evolutionary path to terminal differentiation and division of labor in cyanobacteria. J Theor Biol 262:23–34

Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S (2007) The Sorcerer II global ocean sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. PLoS Biol 5:398–431

Schindler DW (1977) Evolution of phosphorus limitation in lakes. Science 195:260–262

Schirmer A, Rude MA, Li X, Popova E, del Cardayre SB (2010) Microbial biosynthesis of alkanes. Science 329:559–562

Schirrmeister BE, Antonelli A, Bagheri HC (2011) The origins of multicellularity in cyanobacteria. BMC Evol Biol 11:1–28

Stace CA (2010) Classification by molecules: what's in it for field botanists. Watsonia 28:103–122

Stanier RY, Sistrom WR, Hansen TA, Whitton BA, Castenholz RW, Pfennig N, Gorlenko VN, Kondratieva EN, Eimhjellen KE, Whittenbury R, Gherna RL, Trüper HG (1978) Proposal to place nomenclature of cyanobacteria (blue-green algae) under the rules of the International Code of Nomenclature of Bacteria. Int J Syst Bacteriol 28:335–336

Starkenburg SR, Reitenga KG, Freitas T, Johnson S, Chain PSG, Garcia-Pichel F, Kuske CR (2011) The genome of the cyanobacterium *Microcoleus vaginatus* FGP-2, a photosynthetic ecosystem engineer of arid land soil biocrusts worldwide. J Bacteriol. doi:10.1128/JB.05138-11

Steinberg CEW, Schäfer H, Beisker W, Brüggemann R (1998) Deriving restoration goals for acidified lakes from taxonomic studies. Restor Ecol 6:327–335

Stucken K, John U, Cembella A, Murillo AM, Soto-Liebe K, Fuentes-Valdés JJ, Friedel M, Plominsky AM, Vásquez M, Glöckner G (2010) The smallest known genomes of multicellular and toxic cyanobacteria: comparison, minimal gene sets for linked traits and the evolutionary implications. Plos One 5(2): e9235, 15 pp. doi:10.1371/journal.pone.0009235

Van Donk E, Ianora A, Vos M (2011) Induced defences in marine and freshwater phytoplankton: a review. Hydrobiologia 668:3–19

van Gremberghe I, Leliaert F, Mergeay J, Vanormelingen P, van der Gucht K, Debeer A-E, Lacerot G, De Meester L, Vyverman W (2011) Lack of phylogeographic structure in the freshwater cyanobacterium *Microcystis aeruginosa* suggests global dispersal. Plos One 6:1–12

van Liere L, Walsby AE (1982) Interactions of cyanobacteria with light. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell/University of California Press, Oxford/Berkeley, pp 9–45, 655 pp

Van Mooy BAS, Hmelo LR, Sofen LE, Campagna SR, May AL, Dyhrman ST, Heithoff A, Webb EA, Momper L, Mincer TJ (2012) Quorum sensing control of phosphorus acquisition in *Trichodesmium* consortia. ISME J 6(2):422–429

Wagner F, Falkner G (2001) Phosphate limitation. In: Rai LC, Gaur JP (eds) Algal adaptation to environmental stresses. Springer, Heidelberg/New York, pp 65–110

Waterbury JB, Willey JM, Franks DG, Valois FW, Watson SW (1985) A cyanobacterium capable of swimming motility. Science 230:74–76

Waybright TJ, Terlizzi DE, Ferrier MD (2009) Chemical characterisation of the aqueous algistatic fraction of barley straw (*Hordeum vulgare*) inhibiting *Microcystis aeruginos*a. J Appl Phycol 32:333–340

Willey JM, Waterbury JB (1989) Chemotaxis toward nitrogenous compounds by swimming strains of marine *Synechococcus* spp. Appl Environ Microbiol. 55:1888–1894

Whitton BA (2000) Soils and rice-fields. In: Whitton BA, Potts M (eds) Ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 235–255, 669 pp

Whitton BA (2011) Phylum cyanobacteria (Cyanophyta). In: John DM, Whitton BA, Brook AJ (eds) The freshwater algal flora of the British Isles, 2nd edn. Cambridge University Press, Cambridge, UK, pp 31–158, 878 pp

Whitton BA, Potts M (2000) Introduction to the cyanobacteria. In: Whitton BA, Potts M (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 1–11, 689 pp

Whitton BA, Grainger SLJ, Hawley GRW, Simon JW (1991) Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb Ecol 21:85–98

Whitton BA, Balbi DM, Donaldson A (2003) Blue-Green Algae of the British Isles Interactive key to the species. CD-ROM School of Biological and Biomedical Sciences, University of Durham, Durham, UK

Whitton BA, Al-Shehri AH, Ellwood NTW, Turner BL (2005) Ecological aspects of phosphatase activity in cyanobacteria, eukaryotic algae and bryophytes. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. Commonwealth Agricultural Bureau, Wallingford, UK, pp 205–224, 399 pp. ISBN Organic Phosphorus in the Environment

Wolk CP (1983) Heterocysts. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell/University of California Press, Oxford/Berkeley, pp 359–386, 688 pp

Wolk CP, Ernst A, Elhai J (1994) Heterocyst metabolism and development. In: Bryant DA (ed) The Molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp 769–823, 669 pp

Wu Y, Liu J, Yang L, Chen Hm Zhang S, Zhao J, Zhang N (2011) Allelopathic control of cyanobacterial blooms by periphyton biofilms. Environ Microbiol 13(3):604–615

Zapomělová E, Jezberová J, Hrouzek P, Hisem D, Řeháková K, Komárková J (2009) Polyphasic characterization of three strains of *Anabaena reniformis* and *Aphanizomenon aphanizomenoides* to *Sphaerospermum* gen. nov. (incl *Anabaena kisseleviana*). J Phycol 45:1363–1373

Zehr JP, Bench SR, Carter BJ, Hewson I, Niazi F, Shi T, Tripp HJ, Affourit JP (2008) Globally distributed uncultivated oceanic N_2-fixing cyanobacteria lack oxygenic photosystem II. Science 322:1110–1112

Zhang S, Bryant DA (2011) The tricarboxylic acid cycle in cyanobacteria. Science 334(6062):1551–1553

Zhao-Liang X (1984) Investigation on the procaryotic microfossils from Gaoyuzhuang Formation, Jixian, North China. Acta Bot Sin 26(216–222):312–319

Zwirglmaier K, Heywood JL, Chamberlain K, Woodward EMS, Zubkov MV, Scanlan DJ (2007) Basin-scale distribution patterns of picocyanobacterial lineages in the Atlantic Ocean. Environ Microbiol 99:1278–1290

The Fossil Record of Cyanobacteria

2

J. William Schopf

Contents

Abbreviations

BIF	banded iron-formation
CLSM	confocal laser scanning microscopy
GOE	Grand Oxidation Event
Ma	million years
NMR	solid-state ^{13}C nuclear magnetic resonance
RIP	Raman Index of Preservation
Rubisco	ribulose bisphospate carboxylase/oxygenase
XANES	X-ray absorption near-edge spectroscopy

Summary

Fossil evidence of cyanobacteria, represented in the geological record by microbially laminated stromatolites, cyanobacterial and cyanobacterium-like microscopic fossils, and carbon isotopic data consistent with the presence of Rubisco-mediated CO_2-fixation, extends back to ~3,500 million years ago. The most abundant and best-documented fossil cyanobacteria, known from thousands of specimens preserved in several hundred geological units, belong to five taxonomic families: the Oscillatoriaceae, Nostocaceae, Chroococcaceae, Entophysalidaceae and Pleurocapsaceae. As documented by the essentially identical morphologies, life cycles, and ecologic settings of such fossils and their modern counterparts, members of these families have exhibited extreme evolutionary stasis over enormous segments of geological time. Because of the incompleteness of the fossil record, however, such data do not resolve the time of origin of O_2-producing cyanobacteria from their anoxygenic, bacterial, evolutionary precursors. Though it is well established that Earth's ecosystem has included autotrophs since its very early stages, available data indicate only that O_2-producing photoautotrophic cyanobacteria originated earlier than the Great Oxidation Event at ~2,450 million years ago; that such microbes were evidently extant by ~2,700 million years ago; and that the origin of oxygenic photosynthesis may date from as early as, or even earlier than, 3,500 million years ago.

J.W. Schopf (✉)
Department of Earth and Space Sciences, Center for the Study of Evolution and the Origin of Life, Institute of Geophysics and Planetary Physics, Molecular Biology Institute, University of California, Los Angeles, CA 90095-1567, USA
e-mail: schopf@ess.ucla.edu

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2.1 Overview of the Microbial Fossil Record

2.1.1 Introduction

Geological time is divided into two major segments: (1) the *Phanerozoic Eon*, the younger and much shorter of the segments, that begins with the first appearance of shelly invertebrate animals ~542 million years (Ma) ago and includes the familiar evolutionary successions from algae to spore plants and then to seed plants, and from marine invertebrates to fish and then to terrestrial vertebrates; and (2) the *Precambrian Eon*, the longer of the segments that spans the earlier seven-eighths of Earth history, extending from the formation of the planet, ~4,500 Ma ago, to the beginning of the Phanerozoic. The Precambrian, in turn, is subdivided into two exceedingly long segments – each some 2,000 Ma in duration – the *Archean*, extending from the formation of the planet to 2,500 Ma ago, and the *Proterozoic*, spanning the time from 2,500 Ma ago to the beginning of the Phanerozoic. The oldest known fossils date from ~3,500 Ma ago (Schopf 1993, 2006; Schopf et al. 2007; DeGregorio et al. 2009), with hints of life being present in ~3,830-Ma-old rocks, among the oldest known on Earth (Mojzsis et al. 1996; McKeegan et al. 2007).

Though it is likely that the earliest forms of life were heterotrophs, originating within and metabolically dependent on abiotically produced "primordial soup" (Oparin 1938; summarized in Schopf 1999), evidence from the rock record (primarily, microbially produced stromatolites, cellular microscopic fossils and the carbon isotopic composition of preserved organic matter) establishes that photoautotrophy has served as the foundation of the world's ecosystem since at least 3,500 Ma ago. The principal unsolved problem is not whether photosynthesis was an exceedingly ancient evolutionary innovation, but, rather, when did O_2-producing photosynthesis originate, a metabolic process that arose as an evolutionary derivative of a more primitive form of photoautotrophy, anoxygenic photosynthesis, characteristic of non-cyanobacterial photosynthetic bacteria (Blankenship 1992; Blankenship and Hartman 1998). Among all evolutionary innovations, the one which probably had the greatest impact on Earth's ecosystem and subsequent biotic history was the origin of O_2-producing photosynthetic cyanobacteria – dating from the earliest, Archean, segment of geological time. Their advent altered the world's environment forever and provided the biologically useable O_2 required for aerobic respiration, a decidedly more efficient energy-generating process than its anaerobic (fermentative) precursors (Schopf 1999).

The time of origin of this globally altering event can be addressed by answering a single question: "When did cyanobacteria originate?" Firm fossil evidence of the existence of cyanobacteria, the earliest-evolved "complete aerobes" capable of both O_2-producing photosynthesis and O_2-consuming respiration, would establish that the sequence of metabolic innovations that led to their emergence (anaerobic heterotrophy, followed by anaerobic photoautotrophy and then aerobic autotrophy and aerobic respiration) had already evolved, giving rise to an ancient, but metabolically fully modern, ecosystem (Schopf 1996, 1999). Evidence to answer this question should be expected to be preserved in the Precambrian rock record. Stromatolites, microbially layered deposits dominated today by filamentous and coccoid cyanobacteria, are present throughout virtually all of the known geological record; cellularly preserved fossils of cyanobacteria dominate the record of Precambrian life; and rock-derived carbon isotopic data are consistent with the presence of photosynthetic microorganisms back to ~3,500 Ma and possibly to >3,800 Ma ago. Nevertheless, a firm answer to the question of the time of origin is not yet available: the earliest known stromatolites might have been formed by anoxygenic photosynthesizers; the cyanobacterium-like fossils in rocks ~3,200- to 3,500-Ma-old might be remnants of non-O_2-producing microbes; and though a vast amount of carbon isotopic data are consistent with the presence of oxygenic photosynthesis as early as ~3,500 Ma ago, they do not rule out the possibility that the role of primary producer in the world's most ancient ecosystems was played by anaerobic, anoxygenic, photosynthetic bacteria.

It is not surprising that the question of time of origin of cyanobacteria and thus O_2-producing photosynthesis is still unresolved. In contrast to palaeontological studies of the Phanerozoic history of life, the outlines of which were already known in the mid-1800s (Darwin 1859), successful investigation of the earlier, Precambrian, fossil record did not begin until the mid-1960s (Barghoorn and Schopf 1965; Barghoorn and Tyler 1965; Cloud 1965; Schopf 1968). Although much progress has been made during the ensuing decades (e.g. Schopf and Bottjer 2009) in showing that Precambrian microbes were abundant, ubiquitous, metabolically diverse, and biotically predominant, knowledge of the early fossil record remains far from complete. Moreover, due to the "geologic cycle," the repeated sequence of mountain building, erosion, and deposition of the eroded products into sedimentary basins, the average "lifetime" of a geological unit is only some 200 Ma. For this reason, the rock record that has survived to the present rapidly diminishes with increasing geological age, which severely limits the ancient fossil record available for study. About half of the potentially fossil-bearing sedimentary rocks that have survived date from the Phanerozoic (the recent one-eighth of geological time); most of the rest are Precambrian, spanning the earlier seven-eighths of Earth history; Archean-age rocks – those older than 2,500 Ma in which evidence of the earliest oxygenic photosynthesizers is expected to occur – represent only about 5% of the surviving rock mass (Garrels and Mackenzie 1971). Although the known fossil record of cellularly preserved

microbes extends deep into the Precambrian – throughout all of the Proterozoic and much of the Archean, it becomes increasingly sparse and patchy in units older than ~2,000 Ma and the history of the various microbial lineages becomes increasingly difficult to decipher.

2.1.2 The Great Oxidation Event (GOE)

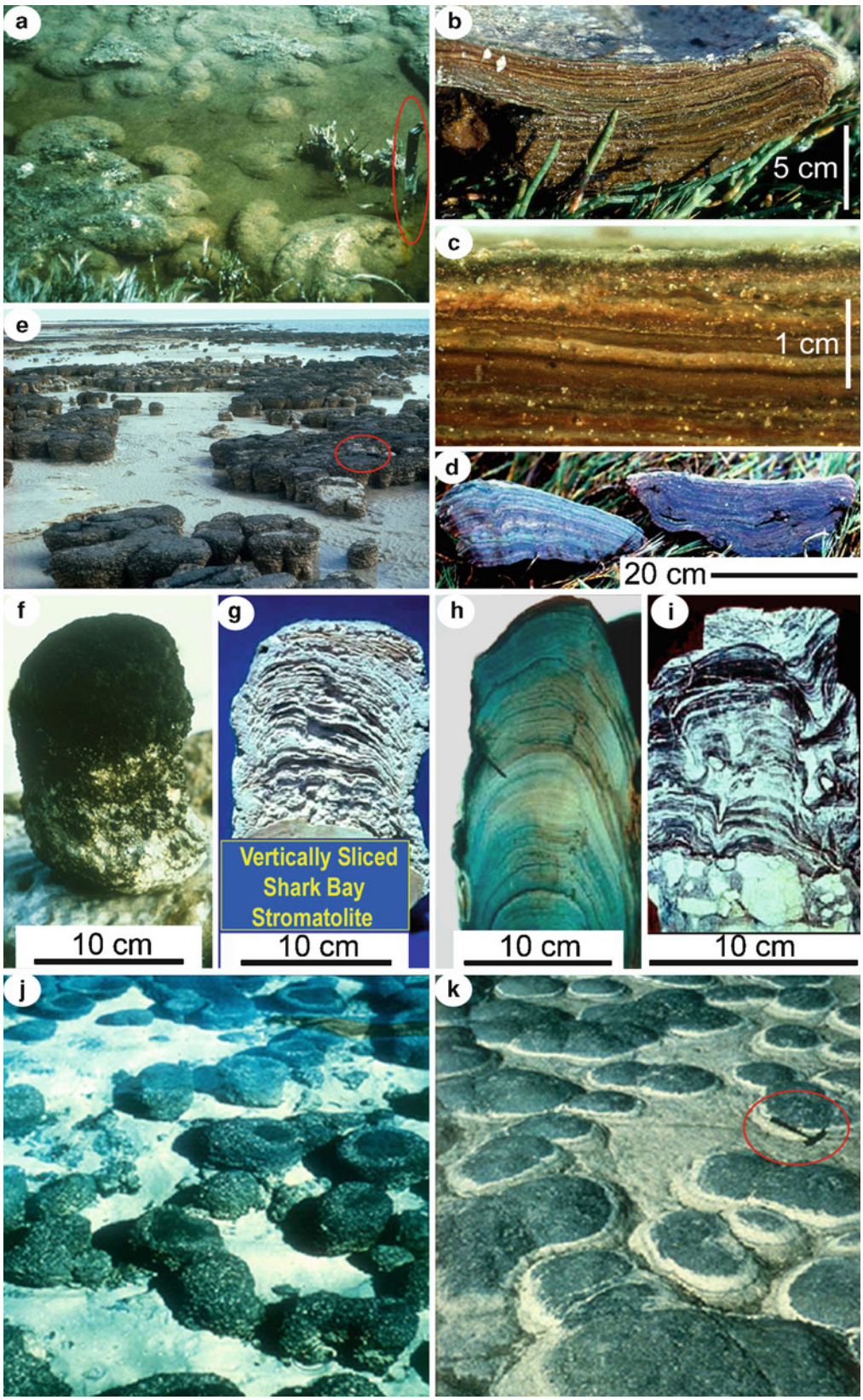
Despite the problems posed by the petering-out of the rock and fossil records over geological time, the records that *have* survived are sufficient to establish the presence of molecular oxygen in the Earth's atmosphere – and, by implication, of cyanobacterial oxygen-producing photoautotrophs – at least as early as ~2,450 Ma ago. As summarized by Holland (2002) and Canfield (2005), from about 2,200 Ma ago to the present, sandstones known as red beds have been deposited on land surfaces by meandering rivers and windblown dust. The beds are coloured red by the presence of the mineral hematite (Fe_2O_3), iron oxide that typically forms a thin veneer on individual quartz sand gains and the presence of which indicates that the atmosphere at the time was oxidizing. In contrast, in numerous terrains older than about 2,400 Ma, conglomeratic rocks occur that contain detrital grains of pyrite and uraninite deposited in shallow-water deltaic settings, minerals that in the presence of molecular oxygen are rapidly converted to their oxidized forms – for pyrite (FeS_2) to the mineral hematite (Fe_2O_3); and for uraninite (UO_2) to its soluble more-oxidized form, UO_4. If there had been appreciable oxygen in the overlying atmosphere when these shallow-water sediments were laid down, hematite, rather than pyrite, would occur in such conglomerates and uraninite would have oxidized and been dissolved.

The distinctly differing temporal distributions of red beds and of pyritic uraniferous conglomerates indicates that there was an increase in the amount of oxygen in Earth's atmosphere some 2,200–2,400 Ma ago, a date that has recently been more firmly set by studies of sulphur isotopic ratios preserved in the rock record that evidence a major rise in atmospheric O_2-content at ~2,450 Ma ago (Farquhar et al. 2000, 2007). Since photosynthesis produces well over 99% of the oxygen in the atmosphere, and since no other large-scale source of free oxygen is known, this increase of atmospheric O_2 can be firmly attributed to the activities of cyanobacterial oxygenic photosynthesizers. Nevertheless, the timing of this major increase, dubbed the Great Oxidation Event (Holland 2002), sets only a minimum age of ~2,450 Ma for the presence of these O_2-producing microbes. Because Earth's primordial environment was anoxic, the molecular oxygen generated by the earliest cyanobacteria would have been rapidly sequestered, removed from the atmosphere by its reaction with previously unoxidized substrates (e.g. volcanic gases, unoxidized minerals, and massive amounts of ferrous iron dissolved in the world's oceans) to be "sponged-up" and buried in rock-forming minerals such as the hematitic iron-oxides of banded iron-formations (BIFs) that are globally abundant in geological sequences older than 2,500 Ma. Only after all such substrates had been more or less completely oxidized – after the "rusting of the Earth" had drawn to close – could the oxygen content of Earth's atmosphere have permanently increased, a time lag from the origin of cyanobacterial O_2-producing photosynthesizers that evidently lasted for many hundreds of millions of years.

Three principal lines of evidence can be used to assess the fossil record of cyanobacteria and to address the closely related question of the time of origin of oxygenic photosynthesis – stromatolites, cellular microfossils, and the chemistry of ancient organic matter – each of which is discussed in turn below. Taken as a whole, the evidence indicates that O_2-producing cyanobacteria were extant earlier than 2,450 Ma ago; that such microbes had originated by 2,700 Ma ago; and that the origin of oxygenic photosynthesis may date from as early as, or even earlier than, 3,500 Ma ago.

2.2 Microbial Stromatolites

As preserved in the geological record, stromatolites are finely layered rock structures, typically composed of carbonate minerals (e.g. calcite, $CaCO_3$), that formed by the microbially mediated accretion of laminae, layer upon layer, from the surface of an ancient seafloor or lake bottom. Their mode of formation has been well documented by studies of modern stromatolites, structures known to microbiologists (including those specializing in studies of cyanobacteria) as "microbial mats." The layered organization of such structures reflects the photosynthetic metabolism of the mat-building and stromatolite-forming microorganisms. Thin (mm-thick) mats composed of such microbes formed as the microorganisms multiplied and spread across surfaces that were typically intermittently veneered by detrital or precipitated mineral grains that blocked sunlight. To maintain photosynthesis, mobile members of such communities, such as gliding oscillatoriacean cyanobacteria, moved upward through the accumulated mineral matter to establish a new, overlying, microbial mat. The repeated accretion and subsequent lithification of such mats, augmented commonly by an influx of non-mobile microbes (such as colonial chroococcacean, entophysalidacean, and pleurocapsacean cyanobacteria), can result in the formation of geologically preservable stromatolitic structures that range from small millimetric pustular mounds and columns to large, decimetric, bioherms. In relatively rare instances, during diagenesis (the series of changes that lead to the lithification and geological preservation of such structures), silica from ground water, precipitated as the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Vertically Sliced
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10 cm
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k

mineral quartz (SiO_2), replaces the initially formed carbonate matrix. If replacement occurs early in the history of a deposit, before the mat-building microorganisms decay and disintegrate, cellularly intact microbes can be preserved. However, the vast majority of fossil stromatolites, unaltered by such replacement, are devoid of cellularly preserved microbes: during diagenesis, carbonate grain growth crushes and obliterates the stromatolite-forming microorganisms, leaving only an amorphous thin coaly residuum of microbe-derived carbonaceous matter.

Cyanobacterium-dominated microbial mat communities, living analogues of those that produced the stromatolites of the fossil record, are known today. One such example, from Baja, Mexico, is shown in Fig. 2.1a through c and compared, in Fig. 2.1d, with a similarly laminated fossilized stromatolite ~1,300 Ma in age. Among the best known and most studied lithified modern stromatolites are those shown in Fig. 2.1e through g, carbonate microbial stromatolites that in size, shape, and laminar structure are much like those known from the Precambrian (compare Fig. 2.1g with h and i, and j and k). Such modern stromatolites are usually restricted to refugia, settings such as hot springs and hypersaline lagoons (Fig. 2.1a–g, j) in which the slow-growing microbial mats are not disrupted by grazing and burrowing metazoans. For this reason, stromatolites are not particularly abundant in sediments of the Phanerozoic, deposits laid down in environments dominated by diverse metazoans. However, in the absence of grazing and burrowing animals, as was the situation until the very end of the Precambrian, stromatolites were abundant worldwide in photic-zone carbonate-depositing settings. Known earliest from rocks ~3,500 Ma in age, their distribution over time parallels that of the surviving Precambrian rock record – that is, stromatolite-bearing rock units gradually become decreasingly abundant as the rock record gradually peters out (Fig. 2.2). Such structures establish the presence of flourishing photosynthesis-based microbial communities, but only rarely do they preserve the cellular fossils that might evidence whether the stromatolite-building photoautotrophs were oxygenic, like cyanobacteria, or anoxygenic, like photosynthetic bacteria.

2.2.1 Archean Stromatolites

As is shown in Fig. 2.2, an impressive number of Archean-age geological units – of particular interest because of their potential bearing on the time of origin of cyanobacteria – are known to contain microbially produced stromatolites. Shown in Fig. 2.3 are representative examples: carbonate sediments of the ~2,723-Ma-old Fortescue Group of Western Australia contain domical, pseudocolumnar and branching stromatolites (Fig. 2.3a, b); those of the ~2,985-Ma-old Insuzi Group of South Africa include stratiform and conical forms (Fig. 2.3c, d); and those of the ~3,388-Ma-old Strelley Pool Chert of Western Australia contain domical (Fig. 2.3e), stratiform (Fig. 2.3f) and close-packed conical stromatolites patchily distributed over many tens of square-kilometers (Fig. 2.3g–i). The presence of conical stromatolites in such deposits – like those shown in Fig. 2.3c, d, and g through i and reported from 17 of the 48 units listed in Fig. 2.2 (Hofmann et al. 1999; Hofmann 2000; Allwood et al. 2006; Schopf 2006) – is particularly noteworthy since such distinctive structures cone-shaped structures evidently require for their formation "highly motile mat builders" such as oscillatoriacean cyanobacteria (Grotzinger and Knoll 1999, pp. 342–343).

2.3 Cellular Microbial Fossils

Two principal processes preserve organic-walled cyanobacterial fossils: compression and permineralization. Compression-preserved microorganisms occur in fine-grained detrital sediments such as shales and siltstones, pressed and flattened along bedding planes as the sediment lithified. Although such carbonaceous compression-preserved microbes are poorly known from the Phanerozoic, largely neglected by Phanerozoic palaeontologists who focus chiefly on megascopic fossilized remains, they are appreciably better documented in the Precambrian (e.g. Butterfield 2009).

The microbial fossil record is best known from microorganisms preserved by permineralization. Of all modes of fossil preservation, this process (known also as petrification)

Fig. 2.1 Modern and fossil stromatolites: (**a**–**c**) Modern unlithified stromatolites (microbial mats) at Laguna Figueroa (Laguna Mormona), Baja, Mexico: (**a**) mound-shaped stromatolites (machete, at *right*, for scale); (**b**) vertically sectioned stromatolite comprised of stacked, laterally continuous, microbial mats; (**c**) uppermost part of the specimen in (**b**) showing the cyanobacterium-dominated growth surface (*green layer*, at *top*) and an immediately underlying *pinkish layer* populated by purple photosynthetic bacteria. (**d**) The vertically sectioned modern stromatolite in (**b**), at *right*, for comparison with a vertically sectioned fossil carbonate stromatolite (*left*) from the ~1,300-Ma-old Belt Supergroup of Montana, USA. (**e**) Modern lithified (carbonate) columnar and domical stromatolites at Shark Bay (Hamelin Pool), Western Australia (geologic hammer, at *right*, for scale), exposed at low tide. (**f**, **g**) Modern Shark Bay columnar stromatolite, in (**f**) showing its cyanobacterium-coated growth surface and, in a vertical section of this specimen shown in (**g**), its lithified, upwardly accreted, microbially produced internal layers. (**h**, **i**) For comparison with the lithified modern stromatolite in (**f**) and (**g**), vertically sectioned fossil stromatolites from the ~1,300-Ma-old Belt Supergroup of Montana, USA (**h**) and the ~3,350-Ma-old Fig Tree Group of the eastern Transvaal, South Africa (**i**). (**j**, **k**) Modern lithified Shark Bay stromatolites (**j**) for comparison with (**k**) fossil carbonate stromatolites from the ~2,300-Ma-old Transvaal Dolomite, Cape Province, South Africa; the scale for (**j**) and (**k**) is shown by the *red-circled* geological hammer in (**k**)

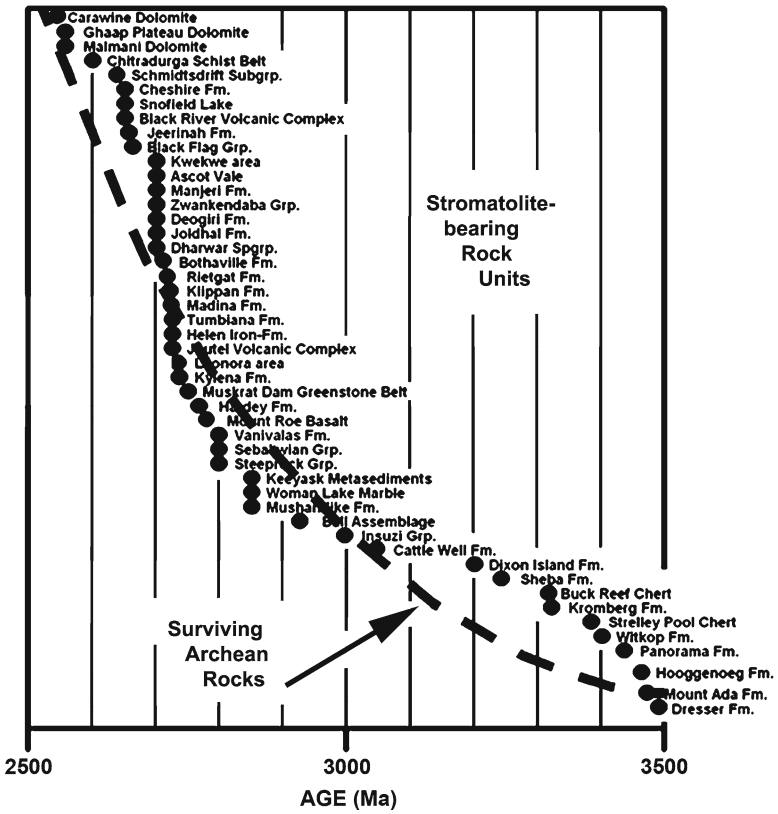


Fig. 2.2 Comparison of temporal distribution of the 48 Archean-age stromatolite-bearing rock units now known (●) with that of Archean-aged rocks that have survived to the present (*dashed line*) (Data from Hofmann 2000; Schopf 2006)

provides the most faithful representation of life-like morphology. Such preservation results from the pervasion of mineral-charged solutions into cells during the early stages of diagenesis, prior to their decay and disintegration. The permeating fluids infill microscopic voids – replacing the watery milieu of the cellular components – to produce a mineral-infused inorganic–organic mix that preserves physically robust structures such as organic-rich cell walls. As a result, both the organismal morphology and cellular anatomy of such fossils can be preserved in three-dimensional microscopic detail. The most common such permineralizing matrix is silica, fine-grained (cryptocrystalline) quartz and the mineral that comprises the rock-type known as chert. Hundreds of microbe-preserving cherts are now known from the Precambrian when silica was abundant in the world's oceans, well before the Phanerozoic appearance of silica-biomineralized sponges, diatoms and radiolarians that today regulate the oceanic silica budget. As shown here, such cherts can contain exquisitely preserved fossil microbes.

2.3.1 Modern and Fossil Cyanobacteria

Microbial classifications based primarily on biomolecular data (e.g. rRNA-, genome-, and DNA base-compositions) are for many modern microbial taxa consistent with traditional morphology-based groupings. For example, cyanobacteria have been assigned on the basis of their organismal structure and pattern of development to five "sections" (cf. morphology-defined taxonomic families) that for many genera mesh well with biochemically based classifications (Herdman et al. 1979a, b; Rippka et al. 1979). Section I (cf. Chroococcaceae) is comprised of predominantly spheroidal, solitary and colonial unicellular cyanobacteria that reproduce by fission or by budding (e.g. *Gloeocapsa*). Section II (cf. Pleurocapsaceae) consists of unicellular or pseudofilamentous forms that by multiple fission give rise to small daughter cells known as baeocytes (e.g. *Pleurocapsa*). Section III (cf. Oscillatoriaceae) encompasses uniseriate cyanobacterial filaments that lack cellular differentiation (e.g. *Oscillatoria* and *Spirulina*). Section IV (cf. Nostocaceae) includes simple uniseriate filaments that exhibit cellular differentiation into akinetes and heterocysts (e.g. *Nostoc*). Section V (cf. Stigonemataceae) is composed of morphologically more complex heterocystous cyanobacterial filaments that exhibit true branching. Representatives of all five groups are known from the fossil record, Sections I–IV dating from well into the Precambrian, whereas representatives of Section V are known earliest from *Stigonema*-like fossils of ~400-Ma-old Rhynie Chert of Scotland (Kidston and Lang 1922). In general, taxa included in Sections II, IV and V are consistent with biochemically-based phylogenies, whereas the cyanobacteria of Sections I and III may not comprise monophyletic lineages. However, because the biochemical

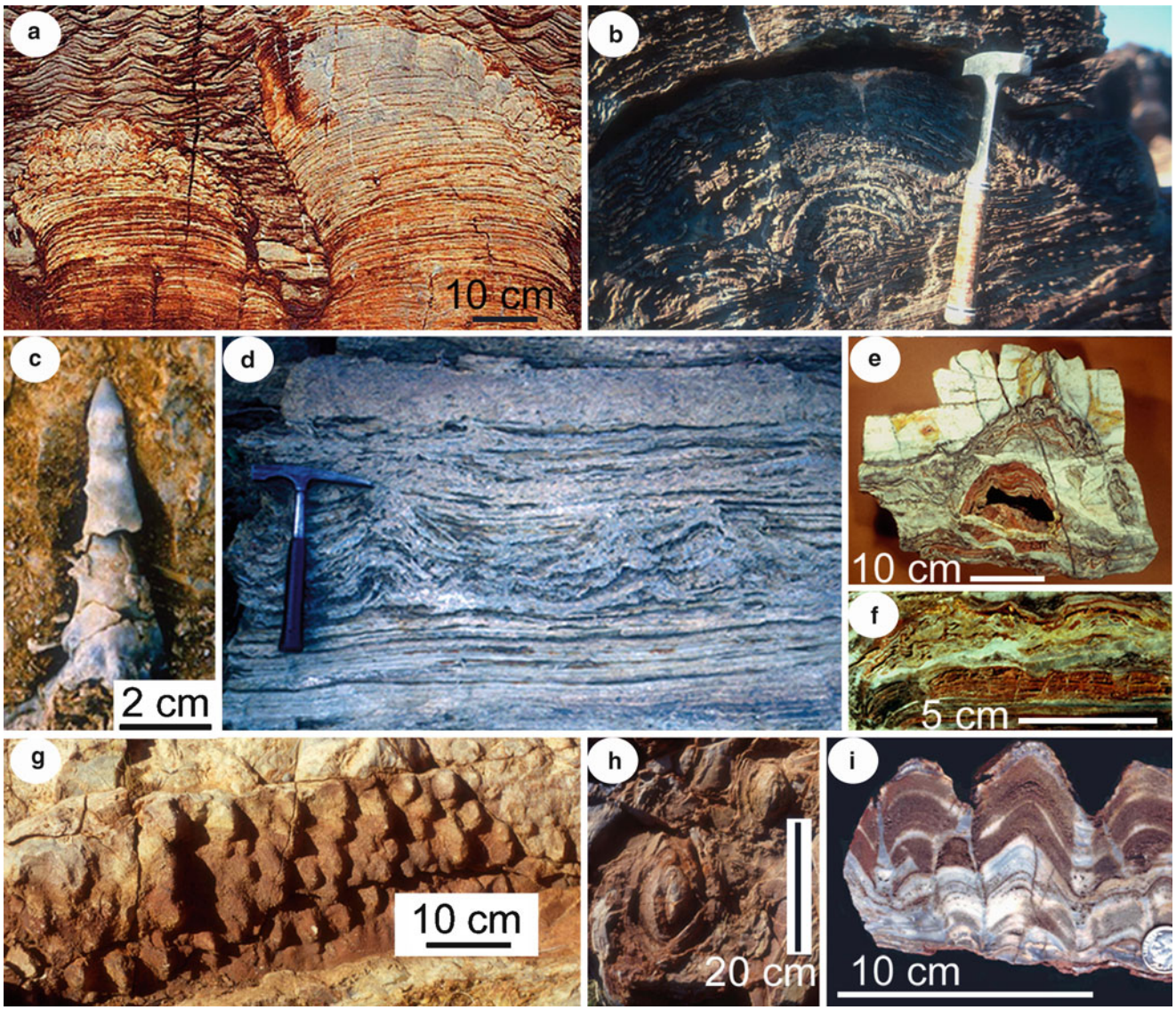


Fig. 2.3 Archean-age microbially laminated stromatolites: (**a**) Domical, pseudocolumnar and branching stromatolites, overlain by rippled sediments, and (**b**) a domical stromatolite from the ~2,723-Ma-old Tumbiana Formation (Fortescue Group) of Western Australia. (**c**) Conical stromatolite and (**d**) stratiform and conical stromatolites from the ~2,985-Ma-old Insuzi Group, South Africa. (**e–i**) Domical (**e**), stratiform (**f**), and laterally linked conical stromatolites (**g** through **i**) from the ~3,388-Ma-old Strelley Pool Chert of Western Australia

components of such microbes, like those of all living systems, are geochemically labile – converted over geological time to coaly kerogen, a geochemically stable complex mix of interlinked polycyclic aromatic hydrocarbons – the classification of cyanobacterial fossils is necessarily based on their morphology, not on their original biochemistry.

2.3.2 Identification of the Major Fossil Types

Four major categories of prokaryotic microbes are known from the fossil record: (1) cyanobacteria; (2) prokaryotes of uncertain systematic relations; (3) sulphate-reducing bacteria; and (4) methane-producing archaeans. Of these, the fossils of uncertain relations (viz., Prokaryotes *Incertae Sedis*) are all regarded as members of the Bacterial rather than the Archaeal Domain, the uncertainty of their systematic position reflecting their morphological similarity both to cyanobacteria and to members of noncyanobacterial bacterial groups. The sulphate-reducers and the methane-producers are known only from isotopic evidence, not from morphologically preserved cellular fossils. Of the four categories, cyanobacteria have the best-documented fossil record, known from thousands of specimens cellularly preserved in hundreds of geological units. Many such fossils are indistinguishable from members of extant cyanobacterial taxa, not only in their morphology

and cellular structure but also in their life cycles and inferred processes of cell division as well as their ecological setting, the biotic structure of the communities in which they occur, and the types of stromatolites they produce.

In comparison with other prokaryotes, whether bacterial or archaeal, most cyanobacteria have somewhat larger cells and more complex morphology. Because of their light-requiring photosynthetic metabolism, cyanobacteria occupy the uppermost surface of microbial mats, rather than lower regions of such biocoenoses where decay and cellular disintegration are prevalent. For this reason, cyanobacteria have a higher probability of becoming incorporated in the fossil record as cellularly intact specimens than do other prokaryotes, especially if they are preserved by permineralization during the early stages of sediment lithification. Of the various morphological components of cyanobacteria, extracellular sheaths and envelopes, initially composed largely of carbohydrates and relatively resistant to degradation, are the most commonly preserved. Although physically robust and organic-rich, cell walls are somewhat less commonly preserved, and in the cells of fossilized cyanobacterial filaments, the originally peptidoglycan-containing thick lateral walls are more commonly preserved than the thinner peptidoglycan-deficient transverse walls. The intracellular biochemical and structural components of such cells (e.g. ribosomes, proteins, strands of DNA, RNA and the like) are evidently never preserved intact. Not only are such components geochemically unstable (degrading to their monomeric constituents within a few to tens of thousands of years), but also, along with the watery intracellular milieu, such organics are typically leached out of such cells during preservation or, if recombined into kerogen, occur as constituents of small coaly carbonaceous intracellular bodies, collapsed remnants of degraded protoplasm.

2.4 Cyanobacterial Fossils

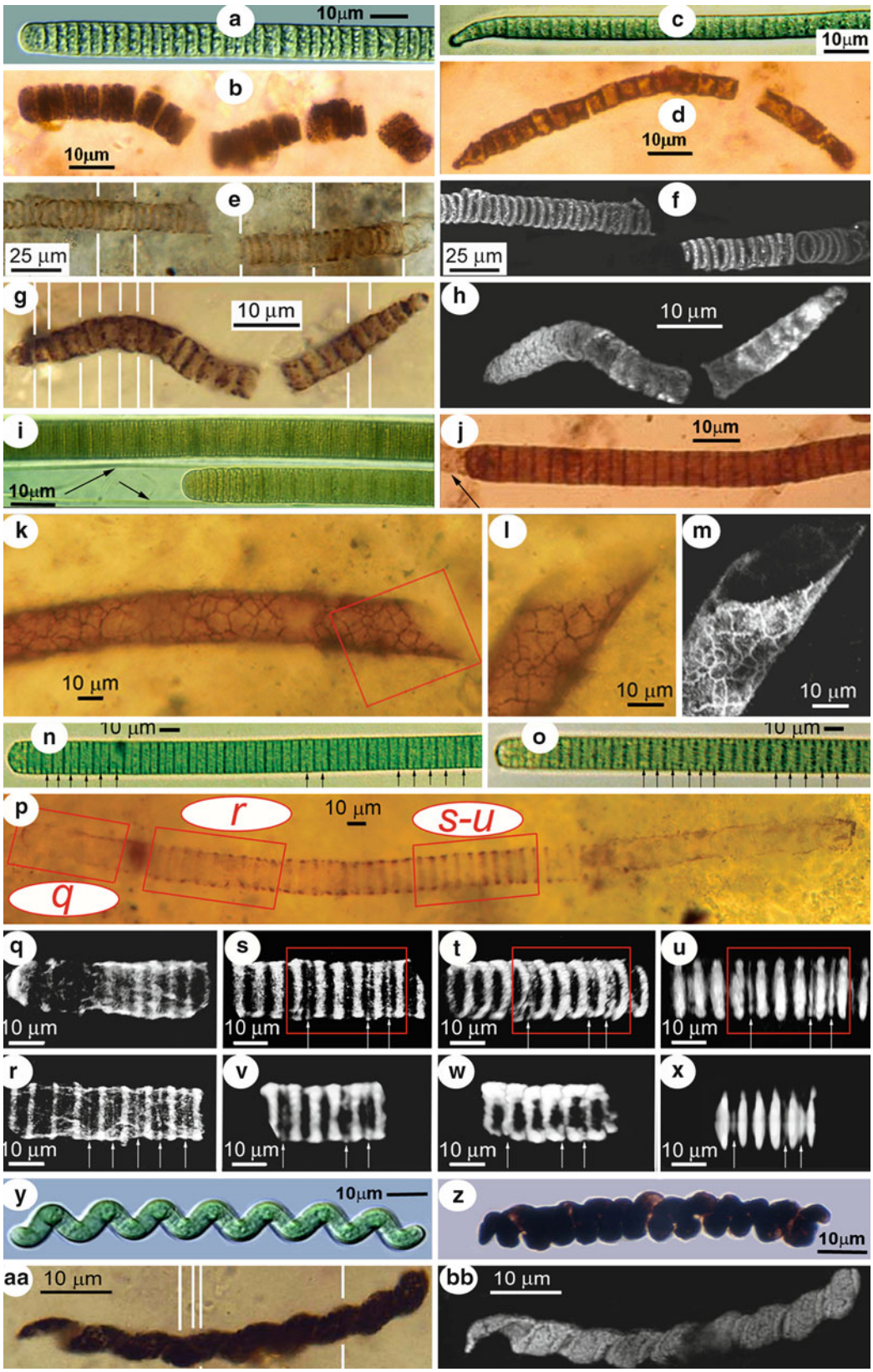
The fossil record of cyanobacteria, summarized here based on specimens preserved in Precambrian-age deposits where the group is well-known and best documented, is composed primarily of oscillatoriacean and nostocacean filaments and of chroococcacean, entophysalidacean and pleurocapsacean coccoid to ellipsoid unicells and colonies. Members of these five cyanobacterial families – each of which is discussed, in turn, below – are the principal components also of modern mat-building microbial stromatolitic communities.

2.4.1 Filamentous Cyanobacteria

2.4.1.1 Oscillatoriaceae

Among the five families of cyanobacteria that are well represented in the fossil record, the Oscillatoriaceae, cyanobacteria characterized by simple unbranched uniseriate trichomes composed of discoidal, equant, or elongate cells, has the most extensive record, represented by diverse fossils in hundreds of ancient microbial communities. Representative Precambrian examples (~775- to ~800-Ma in age) are shown in Fig. 2.4b d, through f, compared with modern morphological analogues (Fig. 2.4a, c). Although the trichomes of many extant oscillatoriaceans maintain a uniform diameter throughout their length (e.g. Fig. 2.4a, i, n, o), some typically

Fig. 2.4 Modern and fossil oscillatoriacean cyanobacteria: Fossil specimens are in petrographic thin sections of stromatolitic cherts from the ~800-Ma-old Bitter Springs Formation of central Australia (**b**, **d**, **g**, **h**, **aa** and **bb**) and the ~775-Ma-old Chichkan Formation of southern Kazakhstan (**e**, **f**, **k** through **m**, and **p** through **x**), and in acid-macerates of siltstones from the ~1,020-Ma-old Lakhanda Formation (**j**) and ~850-Ma-old Miroedikha Formation (**z**), both of Siberia, Russia. (**a, b**) Optical images of modern *Oscillatoria* sp. (**a**) and the morphologically similar fossil *Oscillatoriopsis breviconvexa* (**b**). (**c**, **d**) Optical images of modern *Oscillatoria amoena* (**c**) and its fossil counterpart, *Cephalophytarion grande*. (**e**, **f**) Optical montage (**e**), composed of six micrographs (denoted by the *white lines*) and, in (**d**), a confocal laser scanning microscopy (*CLSM*) image of fossil *Oscillatoriopsis* sp. showing its discoidal medial cells. (**g**, **h**) Optical montage (**g**), composed of ten photomicrographs (denoted by the *white lines*) and a CLSM image (**h**) of a cellular trichome (*Cephalophytarion laticellulosum*) that descends from where it transects the upper surface of the thin section (at the *far right*) to a depth of 20 μm (at the *far left*). (**i**, **j**) Optical images of modern *Lyngbya* sp. and its fossil counterpart, *Paleolyngbya helva; arrows* point to trichome-encompassing tubular organic sheaths. (**k**–**m**) Optical (**k** and **l**) and a CLSM image (**m**) of *Siphonophycus solidum*, the extracellular tubular sheath of an oscillatoriacean cyanobacterium, the *red rectangle* in (**k**) denoting the area imaged in (**l**) and (**m**). (**n**, **o**) Optical images of two specimens of modern *Oscillatoria* sp. showing the rounded terminal cells (*left*), disc-shaped medial cells, and partial septations (*arrows*) characteristic of oscillatoriacean cyanobacteria. (**p**) Optical image of fossil *Oscillatoriopsis media* descending into a thin section at a low angle from *left* to *right*, shown in a photomontage in which the *red rectangles* denote the areas of the trichome shown in CLSM images (**q** through **u**) and three-dimensional Raman images (**v** through **x**). (**q**) The trichome terminus, showing its rounded end-cell and subtending disc-shaped medial cells. (**r**) Part of the trichome situated ~14 μm deeper in the section than the trichome terminus (and ~28 μm below the upper surface of the section) that exhibits partial septations (*arrows*) like those shown in (**n**) and (**o**). (**s**–**u**) A deeper part of the trichome (~39 μm below upper surface of section) that similarly exhibits partial septations (*arrows*), in (**s**) and (**t**) showing the specimen as viewed from above its upper surface [the same perspective as shown in (**p**), but in (**t**) with the trichome tilted slightly to the right to show its interior], and in (**u**) showing the trichome as viewed from its side. (**v**–**x**) Three-dimensional Raman images (acquired in a spectral window centered in the kerogen "G" band at ~1,605 cm^{-1}) showing the kerogenous composition of the trichome and its partial septations: (**v**), the part of specimen denoted by the *red rectangle* in (**s**), as viewed from above the trichome; (**w**), the part denoted in (**t**), titled slightly to the *left*; (**x**), the part denoted in (**u**), showing the specimen from its side. (**y**–**bb**) Optical photomicrographs (**y** through **aa**) and a CLSM image (**bb**) showing modern *Spirulina* (**a**) for comparison with its helically coiled fossil counterparts, a *Spirulina*-like trichome in (**z**) and, in (**aa**) and (**bb**), *Heliconema funiculum,* in (**aa**) shown in montage composed of five photomicrographs (denoted by *white lines*)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taper toward their apices (Fig. 2.4c), tapered trichomes that are also recorded in fossil examples (Fig. 2.4d, g, h). In addition, although many modern oscillatoriacean trichomes lack (or are enclosed by a very thin) extracellular tubular sheath (e.g. *Oscillatoria*: Fig. 2.4a, c, n, o), others, such as *Lyngbya* are encompassed by a prominent mucilaginous sheath (Fig. 2.4i) which, like the cell walls of oscillatoriacean trichomes, can be preserved in fossil specimens (Fig. 2.4j–m).

The trichomes of the great majority of members of the Oscillatoriaceae are characterized by rounded terminal cells, disc-shaped medial cells, and partial septations, incipient cell walls that grow inward to produce daughter cells (arrows in Fig. 2.4n, o). Although in fossil specimens such thin incipient cell walls are rarely evident by optical microscopy, two techniques recently introduced to such studies (Schopf and Kudryavtsev 2005; Schopf et al. 2002, 2005, 2006) – confocal laser scanning microscopy (CLSM) and Raman imagery – can be used to establish their presence. For example, compare the photomicrographs of modern *Oscillatoria* (Fig. 2.4n, o) with that of its fossil equivalent, *Oscillatoriopsis media*, shown in Fig. 2.4p in a thin slice of chert (a ~100-μm-thick petrographic thin section) from the ~775-Ma-old Chichkan Formation of southern Kazakhstan. Because of the CLSM detectable laser-induced fluorescence of the coaly kerogen (primarily, interlinked polycyclic aromatic hydrocarbons) that comprises the cell walls of the fossil, its detailed cellular structure is appreciably better defined in the CLSM images (Fig. 2.4q–u) than in the corresponding optical image (Fig. 2.4p), whereas 3-D Raman imagery documents the carbonaceous composition of its permineralized (quartz-infused) cells (Fig. 2.4v–x).

The cells of modern oscillatoriaceans divide by the centripetal invagination of partial septations that fuse in the center of a cell to produce transverse cell walls. The lateral cell walls of such trichomes are about twice the thickness of their transverse walls and they contain rigidifying pepdidoglycans that are absent from partial septations and transverse walls except at the cell periphery (Pankratz and Bowen 1963; Frank et al. 1971; Halfen and Castenholz 1971; Drews 1973). Because of these differences, lateral cell walls tend to be relatively well preserved in fossil specimens whereas the thinner transverse walls, like their precursor partial septations, are typically preserved only in part. Despite these differences, use of CLSM to analyze fossil specimens shows the presence of such partial septations (arrows in Fig. 2.4r–u), with 3-D Raman imagery (Fig. 2.4v–u) confirming their carbonaceous composition. Not only do such data establish the oscillatoriacean affinities of such fossil cellular trichomes, showing that they are morphologically essentially identical to living members of the family, but they indicate also that their cell division occurred by the same genetically determined processes as their modern counterparts. Data such as these show that the fossil record of the Oscillatoriaceae extends deep into geological time and that such cyanobacteria have changed little or not at all over thousands of millions of years (Schopf 1994a, 1999, 2009).

In addition to cellular (e.g. *Oscillatoria*) and prominently ensheathed trichomes (e.g. *Lyngbya*), the Oscillatoriaceae includes distinctive spirally wound filaments such as the modern *Spirulina* shown in Fig. 2.4y. As shown in Fig. 2.4z through bb, such *Spirulina*-like helically coiled filaments are known also from the Precambrian fossil record.

2.4.1.2 Nostocaceae

In comparison with the fossil record of oscillatoriaceans, that of similarly filament-forming nostocaceans is poorly known. A characteristic of the Nostocaceae is the presence of intercalary heterocysts, thick-walled cells that permit the nitrogenase enzyme complex of filaments living in a well-oxygenated environment to fix N_2, and which only develop when the organism is deprived of other usable N sources (Schopf 1978). As bacterially generated ammonia is plentiful in stromatolitic microbial communities, neither fossil nor modern filamentous cyanobacteria in them are heterocystous. Such differentiated cells being first known from the Devonian Rhynie chert (Kidston and Lang 1922). Nevertheless, based on organismal and cellular morphology, numerous Precambrian fossils have been assigned to the Nostocaceae. Two such examples are shown in Fig. 2.5b, c,compared with modern *Nostoc* (Fig. 2.5a). Nostocaceans are also represented in the Precambrian record by elongate spore-like cells such as *Archaeoellipsoides* (Horodyski and Donaldson 1980) that date back to ~2,100 Ma ago and closely resemble the reproductive akinetes of extant members of the family (Golubić et al. 1995). One such fossil (*Archaeoellipsoides longus*) is shown in Fig. 2.5d through f. The temporal distribution of the Nostocaceae fits well with the timing of the Great Oxidation Event, ~2,400 Ma ago, before which the nitrogenase-protecting heterocysts of akinete-producing nostocaceans would have been of little selective advantage. Further, rRNA phylogenies indicate that the Nostocaceae, like other heterocystous cyanobacterial families, originated in a burst of evolution well after the appearance of families composed of non-heterocystous coccoid, ellipsoid and filamentous taxa (Giovannoni et al. 1988; Zehr et al. 1997). Like other cyanobacteria, nostocaceans appear to have evolved little or not at all since their origination more than 2,000 million years ago (e.g. compare Fig. 2.5a with b and c).

2.4.2 Coccoid and Ellipsoid Cyanobacteria

2.4.2.1 Chroococcaceae

Figure 2.6 shows numerous specimens of fossil chroococcaceans, characterized by their typical occurrence in envelope-

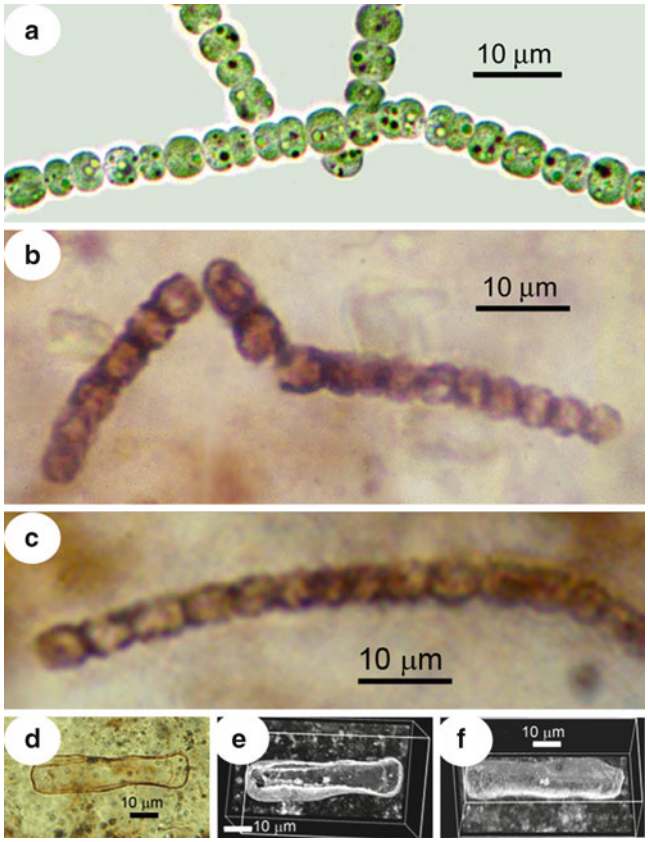


Fig. 2.5 Modern and fossil nostocacean cyanobacteria: (**a**) Modern *Nostoc* PCC 7936 for comparison with two specimens of *Veteronostocale amoenum* (**b**, **c**) shown in a petrographic thin section of stromatolitic chert from the ~800-Ma-old Bitter Springs Formation of central Australia. (**d**–**f**) *Archaeoellipsoides longus*, ankinete characteristic of nostocacean cyanobacteria, from a thin section of the ~775-Ma-old Chichkan Formation of southern Kazakhstan, shown in an optical photomicrograph (**d**) and in (**e**) and (**f**), in confocal laser scanning micrographs: in (**e**), from above the specimen, the same perspective as in (**d**); in (**f**), from below the specimen, showing its smooth lower side

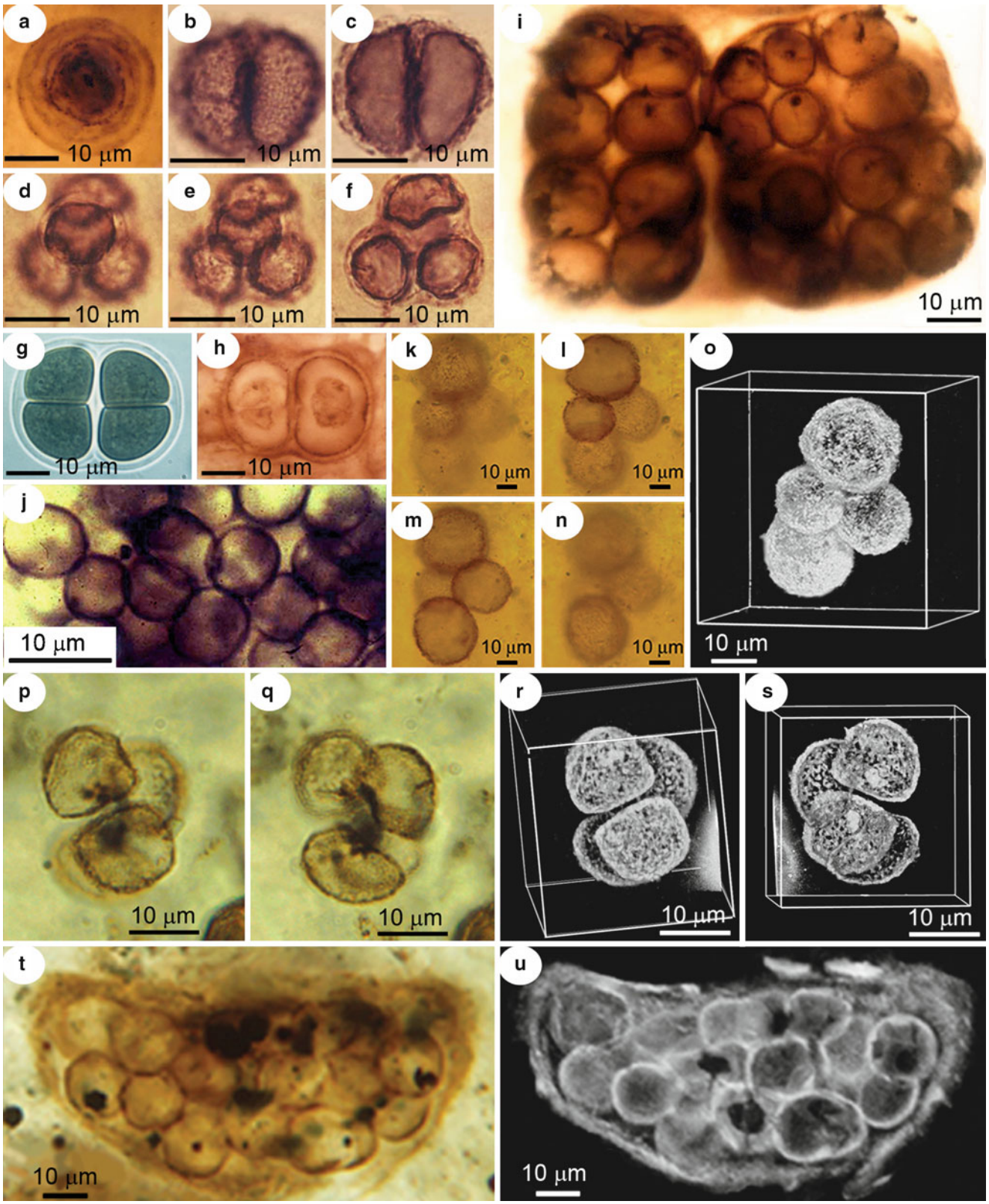
enclosed colonies composed of a few to many coccoid cells. Although chroococcaceans are usually major components of stromatolitic communities, they are almost always of lesser abundance than filamentous mat-building cyanobacteria. Fossils referred to the Chroococcaceae range from isolated single cells, not uncommonly enveloped by multilamellated sheaths (Fig. 2.6a), to pairs (Fig. 2.6b, c) or quartets of sheath-enveloped (Fig. 2.6d–h) or sheath-lacking (Fig. 2.6k–s) spheroidal cells. Some such specimens exhibit a flat-sided "lima bean-shape" (Fig. 2.6b, c, h, p–s) that evidences their formation by cell division like that of modern chroococcaceans, whereas others occur in large aggregates of geometrically ordered (Fig. 2.6i) or irregularly distributed (Fig. 2.6j, t, u) close-packed colonial cells. As shown in Fig. 2.6o, r, s, studies by CLSM provide high-resolution three-dimensional images of such rock-embedded fossils unavailable from standard photomicrography. Virtually all fossil chroococcaceans are similar in salient characteristics (e.g. cell size, cell shape, ensheathed habit, colonial organization, environmental setting) to extant members of the family (compare, for example, Fig. 2.6g, h).

2.4.2.2 Entophysalidaceae

Other fossil cyanobacteria, typically occurring in globose to pustular colonies composed of large numbers of small ellipsoid cells and known from deposits as old as ~2,600 Ma (Altermann and Schopf 1995), are notably similar to modern members of the cyanobacterial family Entophysalidaceae. A rather typical example is shown in Fig. 2.7b, a part of a colony of fossil *Eoentophysalis belcherensis* (Hofmann 1976) preserved in stromatolitic chert of the ~2,100-Ma-old Kasegalik Formation of Canada, compared with living *Entophysalis* (Fig. 2.7a) present in a modern stromatolitic microbial mat community. The similarities between such modern and fossil cyanobacteria are striking: not only are the fossil and modern species morphologically indistinguishable (in cell shape, and in the form and arrangement of originally mucilaginous cell-encompassing envelopes), but they exhibit similar frequency distributions of dividing cells and essentially identical patterns of cell development (resulting from cell division in three perpendicular planes); they form microtexturally similar stromatolitic structures in comparable intertidal to shallow marine environmental settings; they undergo essentially identical postmortem degradation sequences; and they occur in comparable microbial communities, similar both in species composition and in overall diversity (Golubić and Hofmann 1976). Moreover, both in modern and in fossil stromatolites, such entophysalidaceans can be sufficiently abundant to be important mat-formers as shown for a fossil in Fig. 2.7c, stromatolitic layers formed by laterally linked colonies of *Eoentophysalsis* in Kasegalik Formation stromatolites.

2.4.2.3 Pleurocapsaceae

Like entophysalidaceans, baeocyte-producing pleurocapasacean cyanobacteria have an ancient fossil record (Zhang and Golubić 1987). Shown in Fig. 2.8b, for example, is a part of a colony of the Precambrian pleurocapsacean *Paleopleurocapsa reniforma* compared with modern *Pleurocapsa* sp., its living morphological counterpart (Fig. 2.8a). Such fossil and living pleurocapsaceans can be compared in detail. The most studied such example, *Polybessurus bipartitus*, first reported from ~775-Ma-old stromatolites of South Australia (Fairchild 1975; Schopf 1977), is a morphologically distinctive cylindrical fossil pleurocapsacean composed of nested cup-shaped envelopes that extend into long tubular stalks oriented perpendicular to the substrate (Fig. 2.8c–h). Specimens of this taxon in rocks of about the same age from East Greenland have been described as being "a close morphological, reproductive, and behavioral counterpart" to the modern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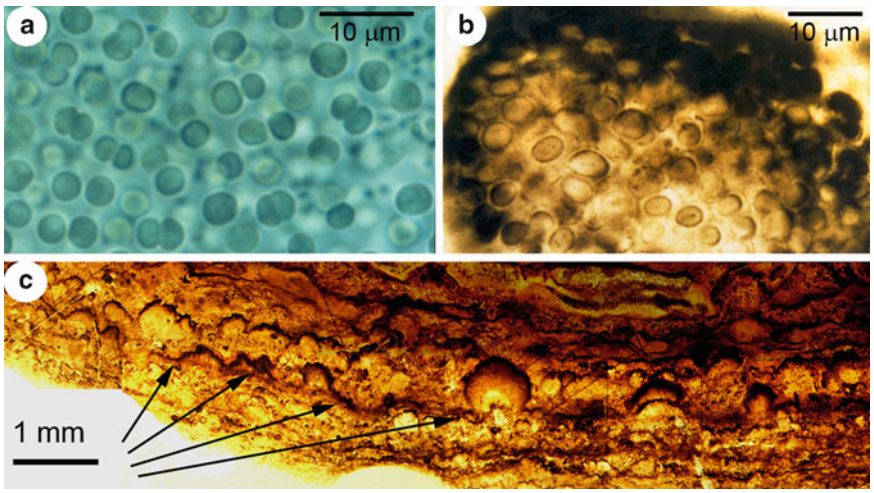

Fig. 2.7 Modern and fossil entophysalidacean cyanobacteria: (**a**) Modern *Entophysalis* sp. for comparison with (**b**) *Eoentophysalis belcherenisis*, in a petrographic thin section of stromatolitic chert of the ~2,100-Ma-old Kasegalik Formation from the Belcher Islands, Canada. (**c**) Low-magnification optical image of pustular stromatolitic laminae formed by laterally interlinked (at *arrows*) entophysalidacean colonies in these stromatolitic cherts

pleurocapsacean *Cyanostylon* present "in Bahamian environments similar to those in which the Proterozoic fossils occur" (Green et al. 1987, p. 928). Another fossil pleurocapsacean (*Palaeopleurocapsa wopfnerii*), described from the ~770-Ma-old Skillogalee Formation of South Australia, has been compared with its living morphological and ecological analogue (*Pleurocapsa fuliginosa*) and interpreted as "further evidence of the evolutionary conservatism of [cyanobacteria]" (Knoll et al. 1975, p. 2492). Two other species of morphologically distinct fossil pleurocapsaceans (the endolithic *Eohyella dichotoma* and *E. rectroclada*), regarded as "compelling examples of the close resemblance between Proterozoic prokaryotes and their modern counterparts" (Knoll et al. 1986, p. 857), have been described from the East Greenland geologic sequence as being "morphologically, developmentally, and behaviorally indistinguishable" from living *Hyella* of the Bahama Banks (Green et al. 1988, pp. 837–838).

2.4.3 Synopsis

Given the foregoing, it is well established that:

- the fossil record of the cyanobacterial lineage extends deep into geological time, to earlier than the GOE at ~2,450 Ma;
- this record, based on thousands of fossils from hundreds of geological units, is particularly well documented for five cyanobacterial families: the Oscillatoriaceae, Nostocaceae, Chroococcaceae, Entophysalidaceae, and Pleurocapsaceae; and
- many members of these families have evidently evolved little or not at all over thousands of millions of years.

One major question has yet to be resolved, namely, When did O_2-producing cyanobacteria first emerge? Evidence from Archean-age (>2,500-Ma-old) rocks may hold the answer. Two key lines of evidence must be considered: (1) the Archean record of microbial fossils and (2) the organic geochemical evidence of ancient microbes preserved in such ancient rocks. Each of these is addressed below.

Fig. 2.6 Modern and fossil chroococcacean cyanobacteria: Fossil specimens are in petrographic thin sections of stromatolitic cherts from the ~1,000-Ma-old Sukhaya Tunguska Formation of Siberia, Russia (**a** and **i**), the ~800-Ma-old Bitter Springs Formation of central Australia (**b** through **f** and **j**), the ~1,500-Ma-old Satka Formation of Bashkiria, Russia (**h**), and the ~775-Ma-old Chichkan Formation of Siberia, Russia (**k** through **u**). **(a–h)** Sheath-enclosed *Gloeocapsa*-like cyanobacteria, spanning a range of morphologies from a multilamellated single cell (**a**), to a sheath-enveloped cell-pair (**b** and **c**), to four-celled sheath-enclosed colonies (**d** through **h**) that illustrate the similarity of modern *Gloeocapsa* sp. (**g**) and its fossil counterpart (*Gloeodiniopsis uralicus*, shown in **h**). (**i**, **j**) Many-celled colonies of fossil chroococcaceans. (**k–o**) 4-celled colony of fossil chroococcaceans showing that, in comparison with optical images obtained at sequentially increasing focal depths (**k** through **n**), appreciably more information can be provided by a single confocal laser scanning microscope (CLSM) image (**o**). (**p–s**) Optical (**p** and **q**) and CLSM (**r** and **s**) images of a four-celled fossil chroococcacean colony of decussate cells (*Myxococcoides dilutus*): (**p**), upper cell pair; (**q**), lower cell pair; (**r**), CLSM image from the perspective shown in (**p**) and (**q**); (**s**), the image shown in (**r**) rotated to show the underside of the decussate tetrad. (**t**, **u**) Optical (**t**) and CLSM (**u**) images of a many-celled fossil chroococcacean (*Myxococcoides inornata*) enveloped by a diaphanous sheath

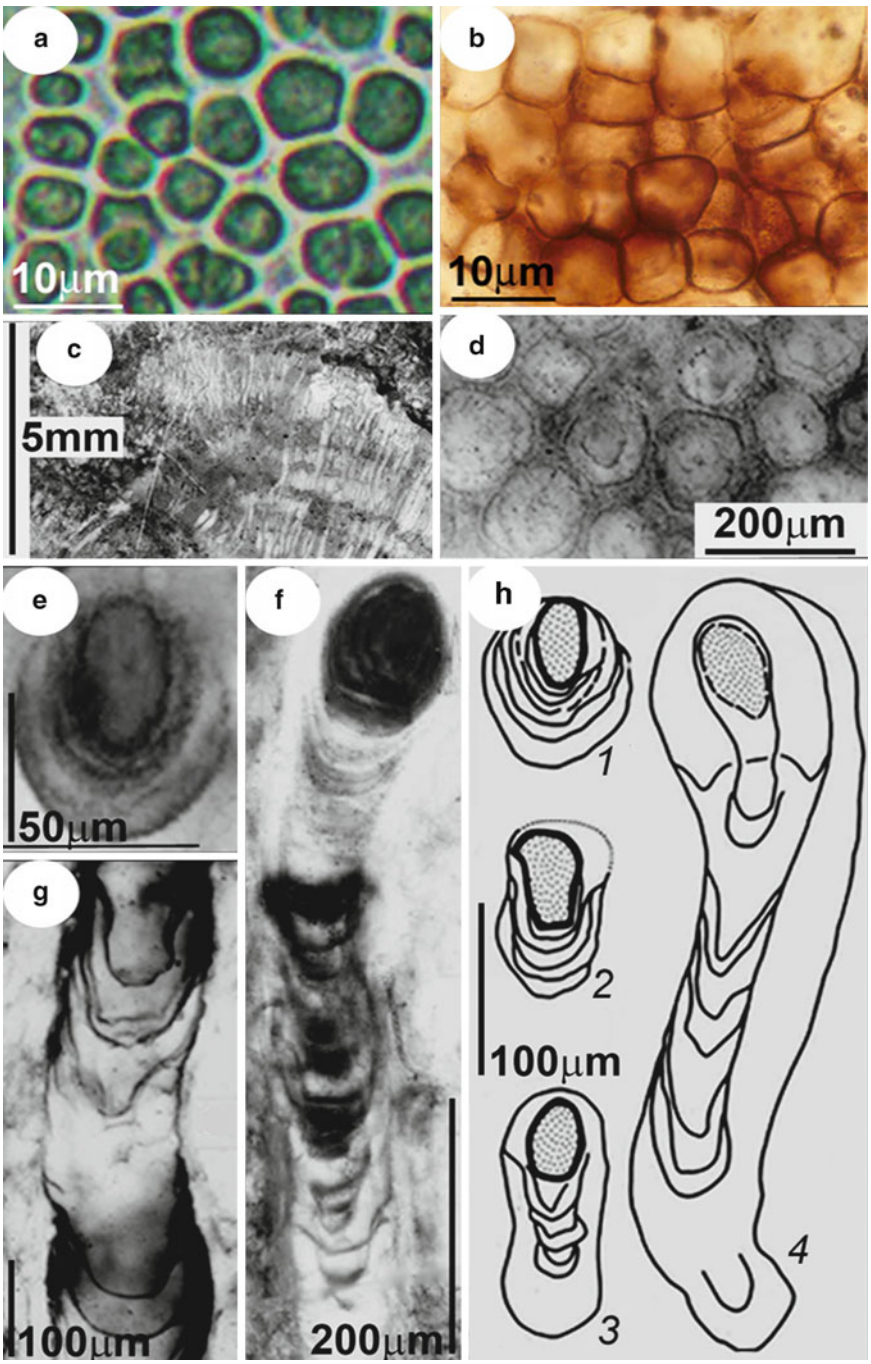

Fig. 2.8 Modern and fossil pleurocapsacean cyanobacteria: (**a**) Modern *Pleurocapsa* sp. (PCC 7327) for comparison with (**b**) *Paleopleurocapsa reniformа* in a petrographic thin section of stromatolitic chert from the ~775-Ma-old Chichkan Formation of southern Kazakhstan. (**c**–**h**) Specimens of the colonial stalk-forming pleurocapsacean cyanobacterium *Polybessurus bipartitus* in petrographic thin sections of stromatolitic chert from the ~775-Ma-old River Wakefield Formation of South Australia: longitudinal (**c**) and transverse sections (**d**) of a colony of pincushion-like vertically oriented and originally mucilaginous extracellular stalks; (**e**) a multilamellated ellipsoid cell at the upper end of a stalk; (**f** and **g**) longitudinal sections of asymmetrically laminated mucilaginous stalks, in (**f**) capped by the ellipsoid stalk-forming cell; (**h**) interpretive drawings (based on tracings of photomicrographs) showing from (*1*) through (*4*) the ontogeny of stalk-formation

2.5 Archean Microbial Fossils

Although cyanobacteria are certain to have been extant by 2,450 Ma ago, and though the O_2-producing photosynthesis that characterizes the group must have originated appreciably earlier, exactly how much earlier remains to be established. Is this uncertainty due to the petering-out over time of the rock record (and the fossil-destroying metamorphic alteration to which the older surviving rocks have been subjected), or, rather, does the fossil record, as now known, evidence the true evolutionary history of the cyanobacterial lineage?

Fossils classed as Bacteria *Incertae Sedis* – that is, fossil prokaryotes of the Bacterial Domain that cannot be referred with certainty to a particular bacterial group – are known throughout the geological record. For virtually all such fossils, the uncertainty in their classification stems from their morphological similarity both to cyanobacteria and to noncyanobacterial bacteria. Such remnants constitute the great majority of

the fossils now known from Archean-age rocks. Because of geologic recycling, only about 5% of rocks exposed at the Earth's surface date from the Archean (Garrels and Mackenzie 1971). Not surprisingly, therefore, the record of Archean microbial fossils is sparse, in the interval between 2,500 and 3,500 Ma reported from only some 40 rock units and comprising only six broad bacterium-like morphotypes (Schopf 2006). Nevertheless, of these geological units, 14 date from the interval between 3,200 and 3,500 million years ago, well evidencing the presence of microbe-level life this early in Earth history.

The Archean fossil microbes most studied are those of the ~3,465-Ma-old Apex chert of northwestern, Western Australia (Schopf 1992a, 1993, 1999; Schopf et al. 2002, 2007). Shown in Fig. 2.9. are specimens of *Primaevifilum conicoterminatum* (Fig. 2.9a, b) and *P. amoenum* (Fig. 2.9c–m), two of 11 taxa of filamentous microorganisms described from this unit (Schopf 1993). These distinctly cellular microscopic fossils, and many of the nine other taxa reported from the deposit, are "cyanobacterium-like" in their morphology and cellular anatomy (e.g. compare Fig. 2.9c–g with Fig. 2.4b, d, g). Nevertheless, because of microbial mimicry – the occurrence of more or less identical morphologies in taxa of oxygenic and non-oxygen-producing microbes (Schopf 1992b, 1999) – organismal and cellular morphology, in and of themselves, cannot provide firm evidence of the physiological capabilities of such very ancient microbes (Schopf 1993). This uncertainty could be resolved were the Archean fossil record, like that of the Proterozoic, sufficiently continuous and well-documented to unambiguously link younger fossils of well-established affinities to their older, and typically less well preserved, evolutionary precursors.

2.6 Organic Geochemical Evidence of Archean Microbes

The existence both of microbially laminated stromatolites and of "cyanobacterium-like" microscopic fossils in rocks dating from ~3,500 Ma ago suggests – but does not establish – that cyanobacteria were extant at this very early stage in Earth history. Rather, such stromatolites and fossils might actually evidence the presence of non-O_2-producing photosynthetic bacteria, evolutionary precursors of the cyanobacterial lineage. In an effort to resolve this question, we will now turn to the data provided by the chemistry of preserved Archean organic matter.

2.6.1 Hydrocarbon Biomarkers

Extraction, isolation, and identification by gas chromatography-mass spectroscopy of organic biomarkers, particularly of various types of hydrocarbons, have provided useful insight into the nature of Precambrian life. For example, identification of the protozoan biomarker tetrahymenol in ~930-Ma-old sediments of the Grand Canyon, Arizona (Summons 1992), supported by the presence of fossils of testate amoebae in the same sedimentary sequence (Bloeser et al. 1977; Bloeser 1985; Schopf 1992c; Porter and Knoll 2000), has established a minimum age for the Proterozoic origin of protozoan protists.

In general, however, such studies have not proven useful to Archean-age deposits. Among the most promising of the few such reports is that of steranes (hydrogenated derivatives of steroids, such as cholesterol) identified in extracts of ~2,700-Ma-old carbonaceous shales of northwestern Australia (Brocks et al. 1999). This finding is unexpected, since steroids occur almost exclusively in eukaryotic cells (e.g. Summons et al. 2006), principally as the components of intracellular membranes, and assured fossil eukaryotes (relatively large-celled spheroidal phytoplankton) are known earliest from sediments ~1,800 Ma in age (Schopf 1992c) that are nearly a billion years younger than the sterane-containing rocks. However, if the reported steranes date from ~2,700 Ma ago, their occurrence would seem to indicate that biologically produced molecular oxygen must have been present in the local environment: steroid biosynthesis involves numerous O_2-requiring enzyme-mediated steps (for cholesterol, 11 such steps, beginning with the cyclization of squalene: Schopf 1978; Summons et al. 2006), and the presence of ~2,700-Ma-old steranes would therefore imply that O_2-producing photosynthesizers must also have been present, since there is no other plausible source for production of the free oxygen required for steroid synthesis.

The interpretation of these reported biomarkers is complicated. Although it seems clear that the sterane-containing shales have been dated correctly, potential contamination from modern sources (e.g. from drilling fluids or introduced during laboratory analyses) is an ever-present problem in such studies. All organic compounds are soluble to some extent in ground water and for this reason can be introduced into rocks long after their deposition, from not only modern but also geologically ancient sources. Moreover, because there are no techniques by which to determine directly the age of organic compounds extracted from ancient sediments, it is difficult to show definitively that such organics are syngenetic with the rock in which they occur. Because of these and related problems, Rasmussen et al. (2008) suggested that the Australian shale-associated steranes are much younger than ~2,700 Ma, most probably less than ~2,200 Ma in age. However, subsequent, more detailed studies that correlate the distribution of these biomarkers with their carbon isotopic compositions and their differing paleoecologic settings provide convincing evidence that they are syngenetic with rocks from which they have been reported (Eigenbrode et al. 2008). And these results showing the syngenicity of such biomarkers with their enclosing sediments have even

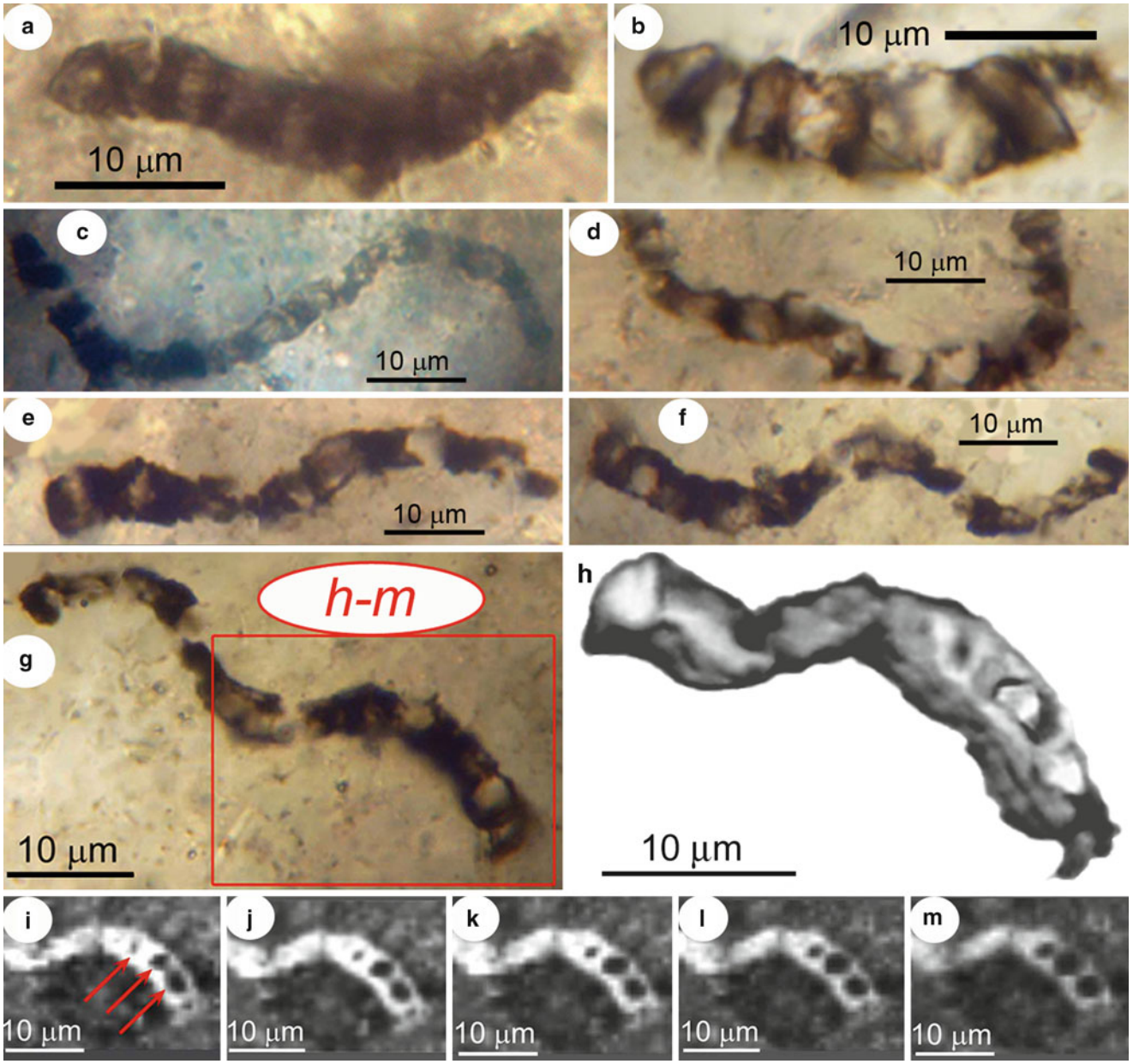

Fig. 2.9 Thin section-embedded filamentous bacteria *Incertae Sedis* (of uncertain systematic position) from the ~3,465-Ma-old Apex chert of northwestern Western Australia; all Raman images (**h** through **m**) were acquired in a spectral window centred in the kerogen "G" band at ~1,605 cm^{-1}. (**a**, **b**) Optical images of two specimens of *Primaevifilum conicoterminatum*, characterized by their discoidal medial cells and conical terminal cells. (**c**–**g**) Optical images of four specimens of *Primaevifilum amoenum*, in (**f**) and (**g**) showing two views of the same specimen situated 3–9 μm *below* the thin section surface; the *red rectangle* in (**g**) denotes the part of the filament shown in (**h**) through (**m**). (**h**) 3=D Raman image; the organic (carbonaceous, kerogenous) filament (*gray*) is cylindrical and, like younger Precambrian chert-embedded cellular fossils (Figs. 2.4b through h and p through x; 2.5b and c; 2.6a through i and h through u; 2.7b; and 2.8b, e and f), is composed of quartz-filled cells (*white*). (**i**–**m**) 2-D Raman images at sequential depths below the filament surface (**i**, at 0.75 μm; **j**, 1.5 μm; **k**, 2.25 μm; **l**, 3.0 μm; **m**, 3.75 μm); *arrows* in (**i**) point to quartz-filled cell lumina (*black*) defined by kerogenous cell walls (*white*), evident also in (**j** through **m**)

more recently been duplicated in studies of essentially the same suite of biomarkers extracted from multiple horizons of South African rock units ~2,600 Ma in age obtained from two boreholes geographically separated by some 24 km (Waldbauer et al. 2009).

Taken together, the available data indicate that sterane biomarkers date to ~2,700 Ma ago, well before the Great Oxidation Event of the early Proterozoic. As such, these biomarkers represent strong presumptive evidence of O_2-producing photoautotrophy during Archean Earth history.

2.6.2 Carbonaceous Kerogen

In contrast to extractable biomarkers, kerogen, the insoluble particulate organic matter of ancient sediments – whether in cherts or shales, and whether occurring as the carbonaceous constituents of cellularly preserved fossils, such as those illustrated here, or as finely divided dispersed coaly particles – is immobile, locked within its embedding rock matrix. In all carbonaceous rocks, whether Phanerozoic or Precambrian and whether or not they contain identifiable fossils, such kerogen occurs entirely or almost entirely as small particles of carbonaceous debris. Because this carbonaceous matter is demonstrably syngenetic with its encompassing mineral matrix, and because it comprises the great bulk of the carbonaceous components of ancient rocks, most analyses of Precambrian organic matter, and virtually all studies of Archean organic matter, have focused on the chemistry of kerogen. Three types of analyses have proved useful: (1) Raman spectroscopy of its molecular structure; (2) solid-state ^{13}C nuclear magnetic resonance (NMR) and X-ray absorption near-edge spectroscopy (XANES) studies of its elemental composition and functional groups; (3) mass spectrometric measurements of its carbon isotopic composition.

As shown in Figs. 2.4v through x and 2.9h through m, 2-D and 3-D Raman imagery provide firm evidence of the carbonaceous (kerogenous) composition of cellular preserved Precambrian microorganisms. Moreover, the Raman spectra on which such images are based can be analyzed to yield the Raman Index of Preservation (RIP) value of the kerogen that comprises the fossils (Schopf et al. 2005). These analyses provide an objective quantitative measure of the geochemical maturity (i.e. fidelity of preservation) of the fossilized organics that is of increasingly widespread use in palaeontology (e.g. Chen et al. 2007; Schopf et al. 2008, 2010; Schopf and Kudryavtsev 2009; Igisu et al. 2009). Thus, the oscillatoriacean trichome from the ~775-Ma-old Chichkan Formation shown in Fig. 2.4p through x has an RIP value of 8.6 (Schopf et al. 2010) indicating that it kerogenous components are only slightly more geochemically altered than those of the especially well-preserved Precambrian cyanobacteria from the ~800 Ma Bitter Springs Formation of central Australia shown in Figs. 2.4b, d, aa, 2.5b, c, and 2.6b through f and j (RIP = 9.0; Schopf et al. 2005). In contrast, the kerogenous cell walls of ~3,465-Ma-old filamentous fossil microbes from the Apex chert of northwestern Western Australia shown in Fig. 2.9a through m are geochemically more mature, having an RIP value of 5.0 (Schopf and Kudryavtsev 2009), but are decidedly less altered than the organic components of cyanobacteria preserved in many other Precambrian deposits (Schopf et al. 2005), such as the highly graphitized stalk-forming pleurocapsaceans from the ~750-Ma-old River Wakefield Formation of South Australia shown in Fig. 2.8c through h (RIP = 1.0; Schopf et al. 2005). Although such data provide strong evidence of the biogenicity of the individual fossils analyzed (Schopf et al. 2008), they do not reveal their physiological characteristics.

Similarly, while studies using ^{13}C nuclear magnetic resonance and X-ray absorption near-edge spectroscopy have provided compelling evidence of the biological origin of the carbonaceous kerogen preserved in ~3,500-Ma-old deposits, they, too, are incapable of demonstrating the presence of O_2-producing cyanobacteria. Two recent publications illustrate the applicability of these techniques. Derenne et al. (2008) used ^{13}C NMR to analyze pyrolysates of kerogen isolated from the ~3,490-Ma-old Towers Formation of northwestern Western Australia by which they documented the presence of aliphatic carbon (CH_2 and CH_3), aromatic C=C (present in the polyaromatic hydrocarbons of which such kerogens are predominantly composed), and C-O and C=O functional groups, and demonstrated also the occurrence of an homologous series of long chain (C_{10}-C_{18}) aliphatic hydrocarbons exhibiting an odd-over-even carbon number predominance, "a unique characteristic of organics formed biologically since it reflects biosynthesis using addition of C2 units" (Derenne et al. 2008, p. 479). DeGregorio et al. (2009) used XANES, backed by other techniques, to establish the biological origin of kerogen in the ~3,465-Ma-old Apex chert, also of northwestern Western Australia and the source of the cellular filamentous Archean microbes illustrated in Fig. 2.9. Their comparative study of the Apex kerogen and that of the well-known microfossil-bearing ~1,900-Ma-old Gunflint chert of southern Ontario, Canada (Barghoorn and Tyler 1965; Cloud 1965), showed that the Apex kerogen contains functional groups – specifically, "carboxyl [–COOH] and phenol [$C_{aromatic}$-OH] peaks" – and that "Apex carbonaceous matter and Gunflint kerogen are chemically complex … [both containing] similar amounts of nitrogen, sulfur, and phosphorous [in which the presence of phosphorus, in particular] implies a biogenic origin" (DeGregorio et al. 2009, p. 632). Like two- and three-dimensional Raman imagery and the Raman spectral analyses discussed above, these studies establish "that the Apex microbe-like features represent authentic biogenic organic matter" (DeGregorio et al. 2009, p. 631) – an important conclusion that lays to rest the claim that the cellular fossils of the Apex chert and the organic matter of which they are composed are of non-biological origin (Brasier et al. 2002). Nevertheless, and again like the Raman data, even such detailed NMR and XANES analyses do not resolve the question of the Early Archean presence of oxygen-producing cyanobacteria.

2.6.3 Carbon Isotopic Evidence of Photosynthesis

Beginning with the pioneering studies of Park and Epstein (1963) and Hoering (1967), data have been amassed from thousands of analyses of the carbon isotopic compositions of inorganic carbonate minerals and carbonaceous kerogens coexisting in Precambrian sediments (e.g. Strauss and Moore 1992). Such data show a consistent difference between the inorganic and organic carbon analyzed in the relative abundances of the two stable isotopes of carbon, ^{12}C and ^{13}C, that extends from the present to ~3,500 Ma ago (Fig. 2.10).

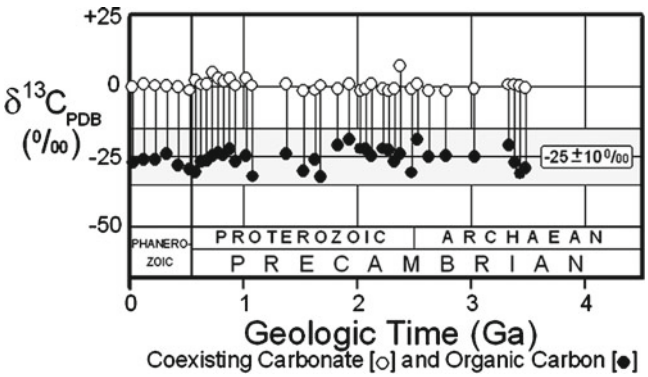

Fig. 2.10 Carbon isotopic values of coexisting carbonate and organic carbon measured in bulk samples of Phanerozoic and Precambrian sedimentary rocks, for the Precambrian represented by data from 100 fossiliferous cherts and shales shown as average values for groups of samples from 50-Ma-long intervals (Strauss and Moore 1992; Schopf 2004)

The enrichment of the fossil organic matter in the lighter isotope, ^{12}C, relative to coexisting carbonate (a proxy for the seawater-dissolved CO_2 required for its precipitation), and the magnitude of the isotopic difference (expressed as $\delta^{13}C_{PDB}$ values) between the inorganic and organic carbon reservoirs that invariably falls within a range of $\delta^{13}C_{PDB}$ values of $25 \pm 10‰$, are consistent with the carbon isotopic fractionation that occurs as a result of Rubisco- (ribulose bisphospate carboxylase/oxygenase-) mediated CO_2-fixation in O_2-producing cyanobacteria (e.g. Hayes et al. 1983, 1992; House et al. 2000, 2003). Such evidence of carbon isotopic fractionation is well documented in rocks ~3,200 to ~3,500 Ma, the oldest fossil-bearing deposits known (Fig. 2.11).

Despite this strong continuous carbon isotopic evidence of photosynthesis, dating to ~3,500 Ma ago, it does not necessarily reflect the Archean presence of cyanobacteria. Because of the mixing of carbonaceous matter from diverse biological sources that occurs as sediments are deposited and the alteration of carbon isotopic compositions that can occur during geological metamorphism, the $\delta^{13}C_{PDB}$ values of the analyzed kerogen range broadly ($\pm 10‰$) and, thus, are consistent not only with primary production by cyanobacteria but by non-O_2-producing photosynthetic bacteria as well. Archean kerogens may have been derived from either or both of these sources. Moreover, interpretation of the data is complicated by the presence in Archean sediments of carbonaceous matter so enriched in ^{12}C as to be plausibly derived only from CH_4-metabolizing methanotrophs, indicating that methane-producing Archaea played a significant role in the ancient ecosystem (Hayes 1983; Schopf 1994b). [As an

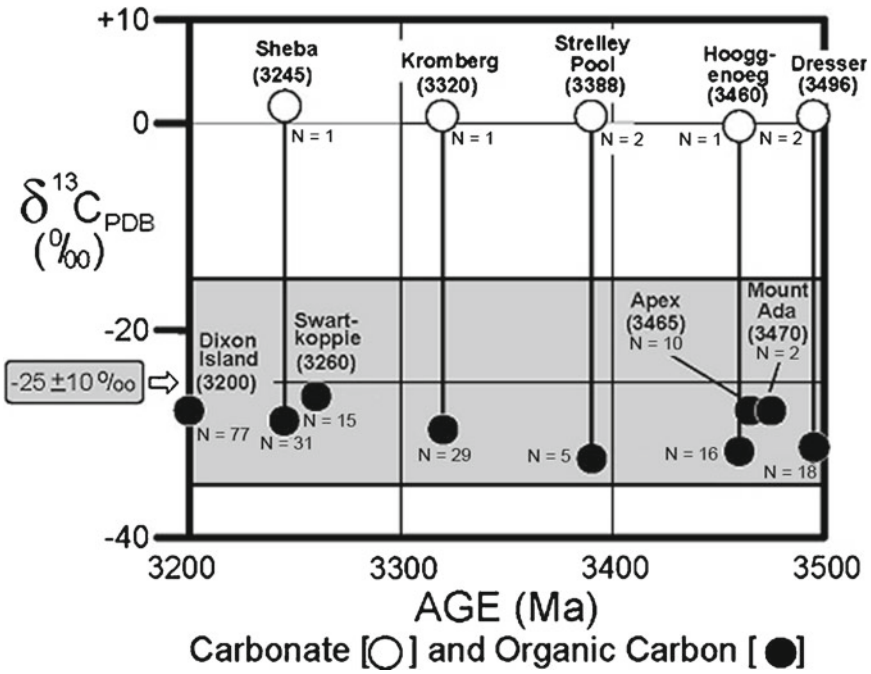

Fig. 2.11 Carbon isotopic values of carbonate and organic carbon measured in bulk samples of nine of the oldest microfossiliferous units known (Schopf 2006)

aside, it should be noted that isotopic analyses of sedimentary pyrite (FeS_2), enriched in S^{32} due to microbial activity, indicate that that sulphate-reducing bacteria were also present in Earth's early biota (Schopf 1999, 2009) and that the presence of ^{12}C-rich graphitic carbon in the oldest sedimentary rocks now known, from Akilia Island off southwestern Greenland, suggests that photosynthetic microbes may have existed as early as ~3,830 million years ago (McKeegan et al. 2007).]

2.7 Conclusions

Microbial communities composed of members of diverse prokaryotic lineages have been extant since early in Earth history (Fig. 2.12). Among such microbes, the most abundant and best documented are cyanobacteria, represented by members of five families: Oscillatoriaceae, Nostocaceae, Chroococcaceae, Entophysalidaceae, Pleurocapsaceae. Perhaps the most striking aspect of the cyanobacterial fossil record is the large body of evidence that documents the evolutionary stasis of diverse members of the group over vast segments of geological time. Such stasis may, or may not be characteristic of microbial lineages generally, but for cyanobacteria it is established firmly by the essentially identical morphologies, life cycles, and ecologic settings exhibited by cyanobacterial fossils and their modern counterparts. In this regard, and despite the fact that cyanobacterial fossils are now known from hundreds of ancient geological units, it is important to note that active studies of the fossil record of the group are of relatively recent vintage and that the documented cyanobacterial fossil record pales in comparison with that of many animal lineages such as trilobites, ammonites, corals, clams, brachiopods, fossils of which have been collected and catalogued for more than two centuries. While much more evidence would be needed to sort out rapid evolution like that typical of eukaryotic plants and animals, even the relatively depauperate cyanobacterial fossil record now known is sufficient to show *lack* of change, maintenance of an evolutionary status quo, over geologically vast periods.

Given the evidence currently available, the times of origin of the various cyanobacterial families can be estimated only approximately (Fig. 2.12) from geological records. Not only is their known fossil record limited by the fact that studies are relatively recent, but their preservation in the rock record, whether as permineralized (petrified) cells in cherty stromatolites or as compressed carbonaceous remnants in shales or siltstones, requires unusual conditions. Moreover, even for more readily preservable organisms such as shelled invertebrate animals the oldest *detected* occurrence of a given lineage is certain to be younger (and for cyanobacteria, perhaps very much younger) than first *actual* occurrences. For truly ancient organisms like cyanobacteria this problem is compounded by the incompleteness of the surviving, potentially fossil-bearing rock record, which becomes increasingly sparse and patchy in units older than ~2,000 Ma, a deficiency particularly acute for rocks of Archean-age (>2,500-Ma-old), which comprise only a miniscule percentage of deposits that have survived to the present. Yet here, too, the available data are sufficient to establish that microbial communities were extant and flourishing early in Earth history: microbially produced stromatolites are known from

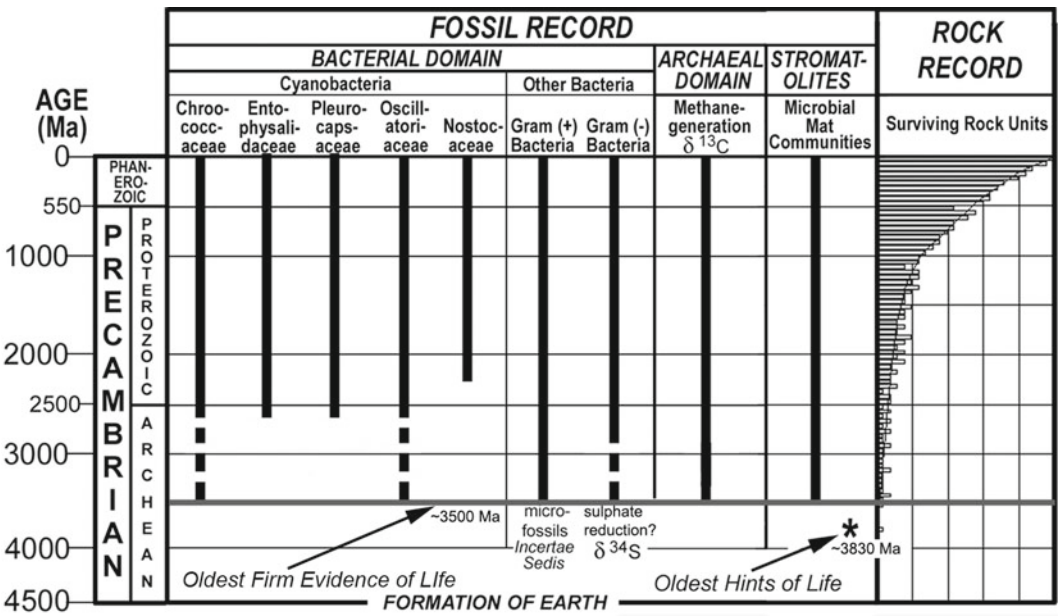

Fig. 2.12 The known fossil record of Bacteria, Archaea, microbially laminated stromatolites, and surviving rock units over geological time, indicating that the oldest firm evidence of life dates from ~3,500 Ma, whereas hints of life are known from rocks ~3,830 Ma in age

48 Archean geological units, of which ten date from between 3,200 and 3,500 Ma ago (Fig. 2.2). *Bona fide* microfossils, comprising six broad bacterium-like morphotypes, have been reported from 40 Archean rock units, of which 14 date from the interval between 3,200 and 3,500 Ma ago (Schopf 2006). Organic geochemical evidence in the form of sterane biomarkers in ~2,700-Ma-old rocks and biogenic carbonaceous kerogen and carbon isotopic evidence in rocks dating to ~3,500 Ma ago well document the presence of microbe-level life early in Earth history. Taken as a whole, the evidence indicates that O_2-producing cyanobacteria originated earlier than the Great Oxidation Event at ~2,450 Ma ago; that such microbes were extant by 2,700 Ma ago; and that the origin of oxygenic photosynthesis may date from as early as, or even earlier than, 3,500 Ma ago.

Regardless of their time of origin, the early evolutionary success of cyanobacteria can be attributed to their photosynthetic production of gaseous oxygen, a toxin lethal to the earlier-evolved strictly anaerobic photosynthetic bacteria with which they initially competed for photosynthetic space (Schopf 1999). Once established, their evolutionary stasis (hypobradytelic rate of evolution; Schopf 1994a) was in part a result of their microscopic size and correspondingly huge populations, their ease of global distribution (by water currents, winds, hurricanes and the like), and their asexual reproduction and the lack of genetic variability it provides. The root of such stasis, however, seems almost certainly to lie in their exceptional ecologic tolerance (Schopf 1994a). Over their exceedingly long evolutionary history, cyanobacteria adapted to the slowly changing global environment – from anoxic to oxygenic, UV-rich to UV-deficient, CO_2-rich to CO_2-deficient, short to increasingly longer day-lengths and, perhaps, from relatively high ambient temperatures (~60°C) to that of the present-day Earth (~15°C). The genomes of cyanobacteria thus encode a history of adaptation unparalleled by virtually any other group of organisms. Throughout their remarkably long-term existence, prokaryotic cyanobacteria have survived and thrived to rank among the most successful forms of life ever to have emerged in life's long history.

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References

Allwood AC, Walter MR, Kamber BS, Marshall CP, Burch IW (2006) Stromatolite reef from the Early Archaean era of Australia. Nature 441:714–718

Altermann W, Schopf JW (1995) Microfossils from the Neoarchean Campbell Group, Griqualand West Sequence of the Transvaal Supergroup, and their paleoenvironmental and evolutionary implications. Precambrian Res 75:65–90

Barghoorn ES, Schopf JW (1965) Microorganisms from the late Precambrian of central Australia. Science 150:337–339

Barghoorn ES, Tyler SA (1965) Microorganisms from the Gunflint chert. Science 147:563–577

Blankenship RE (1992) Origin and early evolution of photosynthesis. Photosynth Res 33:91–111

Blankenship RE, Hartman H (1998) The origin and evolution of oxygenic photosynthesis. Trends Biochem Sci 23:94–97

Bloeser B (1985) *Melanocyrillium*, a new genus of structurally complex late Proterozoic microfossils from the Kwagunt Formation (Chuar Group), Grand Canyon, Arizona. J Paleontol 59:741–765

Bloeser B, Schopf JW, Horodyski RJ, Breed WJ (1977) Chitinozoans from the late Precambrian Chuar Group of the Grand Canyon, Arizona. Science 195:676–679

Brasier MD, Green OR, Jephcoat AP, Kleppe AK, VanKranendonk AJ, Lindsay JF, Steele A, Grassineau NV (2002) Questioning the evidence of Earth's oldest fossils. Nature 416:76–81

Brocks JJ, Logan GA, Buick R, Summons RE (1999) Archean molecular fossils and the early rise of eukaryotes. Science 285:1033–1036

Butterfield NJ (2009) Modes of pre-Ediacaran multicellularity. Precambrian Res 173:201–211

Canfield DE (2005) The early history of atmospheric oxygen: homage to Robert M. Garrels. Annu Rev Earth Planet Sci 33:1–36

Chen JY, Schopf JW, Bottjer DJ, Zhang C-Y, Kudryavtsev AB, Tripathi AB, Wang X-Q, Yang Y-H, Gao X, Yang Y (2007) Raman spectra of a ctenophore embryo from southwestern Shaanxi, China. Proc Natl Acad Sci USA 104:6289–6292

Cloud PE (1965) Significance of the Gunflint (Precambrian) microflora. Science 148:27–45

Darwin CR (1859) On the origin of species by means of natural selection. John Murray, London, 490 pp

DeGregorio BT, Sharp TG, Flynn GJ, Wirick S, Hervig RL (2009) Biogenic origin for Earth's oldest putative fossils. Geology 37:631–634

Derenne S, Robert F, Skrzypczak-Bonduelle A, Gourier D, Binet L, Rouzaud J-N (2008) Molecular evidence for life in the 3.5 billion year old Warrawoona chert. Earth Planet Sci Lett 272:476–480

Drews G (1973) Fine structure and chemical composition of the cell envelopes. In: Carr NG, Whitton BA (eds) The biology of blue-green algae. Blackwell Scientific Publications/University of California Press, Oxford/Berkeley, pp 99–116, 676 pp

Eigenbrode JL, Freeman KH, Summons RE (2008) Methylhopane biomarker hydrocarbons in Hamersley Province sediments provide evidence for Neoarchean aerobiosis. Earth Planet Sci Lett 273:323–331

Fairchild TR (1975) The geologic setting and paleobiology of a Late Precambrian stromatolitic microflora from South Australia. Dissertation, University of California, Los Angeles, 272 pp

Farquhar J, Bao H, Thiemens M (2000) Atmospheric influence of Earth's earliest sulfur cycle. Science 289:756–759

Farquhar J, Peterson M, Johnson DT, Strauss H, Masterson A, Weichert U, Kaufman AJ (2007) Isotopic evidence for Mesoarchaean anoxia and changing atmospheric sulfur chemistry. Nature 449:706–709

Frank H, Lefort M, Martin HH (1971) Elektronenoptische und chemische Untersuchungen an Zellwäden der Blaualgen, *Phormidium unicinatum*. Z Natur B 17:262–268

Garrels RM, Mackenzie FT (1971) Evolution of sedimentary rocks. Norton, New York, 397 pp

Giovannoni SJ, Turner S, Olsen GJ, Barns S, Lane DJ, Pace NR (1988) Evolutionary relationship among cyanobacteria and green chloroplasts. J Bacteriol 170:3584–3592

Golubić S, Hofmann HJ (1976) Comparison of Holocene and mid-Precambrian Entophysalidaceae (Cyanophyta) in stromatolitic mats: cell division and degradation. J Paleontol 50:1074–1082

Golubić S, Sergeev VN, Knoll AH (1995) Mesoproterozoic *Archaeoellipsoides*: akinetes of heterocystous cyanobacteria. Lethaia 28:285–298

Green JW, Knoll AH, Golubić S, Swett K (1987) Paleobiology of distinctive benthic microfossils from the Upper Proterozoic Limestone-Dolomite "Series," central East Greenland. Am J Bot 74:928–940

Green JW, Knoll AH, Swett K (1988) Microfossils from oolites and pisolites of the Upper Proterozoic Eleonore Bay Group, central East Greenland. J Paleontol 62:835–852

Grotzinger JP, Knoll AH (1999) Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? Annu Rev Earth Planet Sci 27:313–358

Halfen LN, Castenholz RW (1971) Gliding motility in the blue-green alga, *Oscillatoria princeps*. J Phycol 7:133–145

Hayes JM (1983) Geochemical evidence bearing on the origin of aerobiosis, a speculative hypothesis. In: Schopf JW (ed) Earth's earliest biosphere, its origin and evolution. Princeton University Press, Princeton, pp 291–301, 543 pp

Hayes JM, Kaplan IR, Wedeking KM (1983) Precambrian organic geochemistry, preservation of the record. In: Schopf JW (ed) Earth's earliest biosphere, its origin and evolution. Princeton University Press, Princeton, pp 93–134, 543 pp

Hayes JM, DesMarais DJ, Lambert IA, Strauss H, Summons RE (1992) Proterozoic biogeochemistry. In: Schopf JW, Klein C (eds) The Proterozoic biosphere, a multidisciplinary study. Cambridge University Press, New York, pp 81–134, 1348 pp

Herdman M, Janvier M, Rippka R, Stanier RY (1979a) Genome size of cyanobacteria. J Gen Microbiol 111:73–85

Herdman M, Janvier M, Waterbury JB, Rippka R, Stanier RY, Mandel M (1979b) Deoxyribonucleic acid base composition of cyanobacteria. J Gen Microbiol 111:63–71

Hoering TC (1967) The organic geochemistry of Precambrian rocks. In: Abelson PH (ed) Researches in geochemistry, vol 2. Wiley, New York, pp 87–111, 663 pp

Hofmann HJ (1976) Precambrian microflora, Belcher Islands, Canada: significance and systematics. J Paleontol 50:1040–1073

Hofmann HJ (2000) Archean stromatolites as microbial archives. In: Riding RE, Awramik SM (eds) Microbial sediments. Springer, Berlin/Heidelberg/New York, pp 315–327, 331 pp

Hofmann HJ, Grey K, Hickman AH, Thorpe RI (1999) Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia. Geol Soc Am Bull 111:1256–1262

Holland HD (2002) Volcanic gases, black smokers, and the Great Oxidation Event. Geochim Cosmochim Acta 66:3811–3826

Horodyski RJ, Donaldson JA (1980) Microfossils from the Middle Proterozoic Dismal Lakes Group, Arctic Canada. Precambrian Res 11:125–159

House CH, Schopf JW, McKeegan KD, Coath CD, Harrison TM, Stetter KO (2000) Carbon isotopic composition of individual Precambrian microfossils. Geology 28:707–710

House CH, Schopf JW, Stetter KO (2003) Carbon isotopic signatures of biochemistry: fractionation by archaeans and other thermophilic prokaryotes. Org Geochem 34:345–356

Igisu M, Ueno Y, Shimojima M, Nakashima S, Awramik SM, Ohta H, Maryuama S (2009) Micro-FTIR spectroscopic signatures of bacterial lipids in Proterozoic microfossils. Precambrian Res 173:19–26

Kidston R, Lang WH (1922) On Old Red Sandstone plants showing structure from the Rhynie chert bed, Aberdeenshire, part V. Trans R Soc Edinb 52:885–902

Knoll AH, Barghoorn ES, Golubić S (1975) *Palaeopleurocapsa wopfnerii* gen. et sp nov., a late-Precambrian blue-green alga and its modern counterpart. Proc Natl Acad Sci USA 72:2488–2492

Knoll AH, Golubić S, Green J, Swett K (1986) Organically preserved microbial endoliths from the late Proterozoic of East Greenland. Nature 321:856–857

McKeegan KD, Kudryavtsev AB, Schopf JW (2007) Raman and ion microscopic imagery of graphite inclusions in apatite from older than 3830 Ma Akilia supracrustal rocks, west Greenland. Geology 35:591–594

Mojzsis S, Arrenhius G, McKeegan KD, Nutman AP, Friend CRL (1996) Evidence for life on Earth before 3,800 million years ago. Nature 384:55–59

Oparin AI (1938) The origin of life. McMillian, New York, 270 pp

Pankratz HS, Bowen CC (1963) Cytology of blue-green algae. I. The cells of Symploca muscorum. Am J Bot 50:387–399

Park R, Epstein S (1963) Carbon isotopic fractionation during photosynthesis. Geochim Cosmochim Acta 21:110–115

Porter SM, Knoll AH (2000) Testate amoebae in the Neoproterozoic Era: evidence from vase-shaped microfossils in the Chuar Group, Grand Canyon. Paleobiology 26:360–385

Rasmussen B, Fletcher IR, Brocks JJ, Kilburn MR (2008) Reassessing the first appearance of eukaryotes and cyanobacteria. Nature 455:1101–1104

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Schopf JW (1968) Microflora of the bitter springs formation, late Precambrian, central Australia. J Paleontol 42:651–688

Schopf JW (1977) Biostratigraphic usefulness of stromatolitic Precambrian microbiotas: a preliminary analysis. Precambrian Res 5:143–173

Schopf JW (1978) The evolution of the earliest cells. Sci Am 239:110–138

Schopf JW (1992a) Paleobiology of the Archean. In: Schopf JW, Klein C (eds) The Proterozoic biosphere, a multidisciplinary study. Cambridge University Press, New York, pp 25–39, 1348 pp

Schopf JW (1992b) Proterozoic prokaryotes: affinities, geologic distribution, and evolutionary trends. In: Schopf JW, Klein C (eds) The Proterozoic biosphere, a multidisciplinary study. Cambridge University Press, New York, pp 195–218, 1348 pp

Schopf JW (1992c) Evolution of the Proterozoic biosphere: benchmarks, tempo, and mode. In: Schopf JW, Klein C (eds) The Proterozoic biosphere, a multidisciplinary study. Cambridge University Press, New York, pp 583–600, 1348 pp

Schopf JW (1993) Microfossils of the Early Archean Apex chert: new evidence of the antiquity of life. Science 260:640–646

Schopf JW (1994a) Disparate rates, differing fates: the rules of evolution changed from the Precambrian to the Phanerozoic. Proc Natl Acad Sci USA 91:6735–6742

Schopf JW (1994b) The oldest known records of life: stromatolites, microfossils, and organic matter from the Early Archean of South Africa and Western Australia. In: Bengtson S (ed) Early life on earth. Columbia University Press, New York, pp 193–206, 656 pp

Schopf JW (1996) Metabolic memories of Earth's earliest biosphere. In: Marshall CR, Schopf JW (eds) Evolution and the molecular revolution. Jones and Bartlett, Boston, pp 73–105

Schopf JW (1999) Cradle of life, the discovery of earth's earliest fossils. Princeton University Press, Princeton, 367 pp

Schopf JW (2004) Geochemical and submicron-scale morphologic analyses of individual Precambrian microorganisms. In: Hill JR, Aizenshtat Z, Baedecker MJ, Claypool G, Eganhouse R, Goldhaber M, Leventhal J, Peters K (eds) Geochemical investigation in Earth and Space Science: a tribute to Isaac R. Kaplan. The Geochemical Society, St. Louis, pp. 365–375, 466 pp

Schopf JW (2006) Fossil evidence of Archaean life. Philos Trans R Soc B 361:869–885

Schopf JW (2009) Paleontology, microbial. In: Lederberg J, Schaechter M (eds) Encyclopedia of microbiology, 3rd edn. Elsevier, Amsterdam, pp 390–400, 4600 pp

Schopf JW, Bottjer DJ (2009) Preface: world summit on ancient microscopic fossils. Precambrian Res 173:1–3

Schopf JW, Kudryavtsev AB (2005) Three-dimensional Raman imagery of Precambrian microscopic organisms. Geobiology 3:1–12

Schopf JW, Kudryavtsev AB (2009) Confocal laser scanning microscopy and Raman imagery of ancient microscopic fossils. Precambrian Res 173:9–49

Schopf JW, Kudryavtsev AB, Agresti DG, Wdowiak TJ, Czaja AD (2002) Laser-Raman imagery of Earth's earliest fossils. Nature 416:73–76

Schopf JW, Kudryavtsev AB, Agresti DG, Czaja AD, Wdowiak TJ (2005) Raman imagery: a new approach to assess the geochemical maturity and biogenicity of permineralized Precambrian fossils. Astrobiology 5:333–371

Schopf JW, Tripathi AB, Kudryavtsev AB (2006) Three-dimensional optical confocal imagery of Precambrian microscopic organisms. Astrobiology 1:1–16

Schopf JW, Kudryavtsev AB, Czaja AD, Tripathi AB (2007) Evidence of Archean life: stromatolites and microfossils. Precambrian Res 158:141–155

Schopf JW, Tewari VC, Kudryavtsev AB (2008) Discovery of a new chert-permineralized microbiota of the Proterozoic Buxa Formation of the Ranjit Window, Sikkim, N.E. India, and its astrobiological implications. Astrobiology 8:735–746

Schopf JW, Kudryavtsev AB, Sergeev VN (2010) Confocal laser scanning microscopy and Raman imagery of the Late Neoproterozoic Chichkan microbiota of South Kazakhstan. J Paleontol 84:402–416

Strauss H, Moore TB (1992) Abundances and isotopic compositions of carbon and sulfur species in whole rock and kerogen samples. In: SchopfJW, Klein C (eds) The Proterozoic biosphere, a multidisciplinary study. Cambridge University Press, New York, pp 709–798, 1348 pp

Summons RE (1992) Abundance and composition of extractable organic matter. In: Schopf JW, Klein C (eds) The Proterozoic biosphere, a multidisciplinary study. Cambridge University Press, New York, pp 101–115, 1348 pp

Summons RE, Bradley AS, Janke LL, Waldbauer JR (2006) Steroids, triterpenoids and molecular oxygen. Philos Trans R Soc B 361:951–968

Waldbauer JR, Sherman LS, Sumner DY, Summons RE (2009) Late Archean molecular fossils from the Transvaal Supergroup record the antiquity of microbial diversity and aerobiosis. Precambrian Res 169:28–47

Zehr JP, Mellon TM, Hiorns WH (1997) Phylogeny of cyanobacterial nifH genes: evolutionary implications and potential applications to natural assemblages. Microbiology 143:1443–1450

Zhang Y, Golubić S (1987) Endolithic microfossils (Cyanophyta) from Early Proterozoic stromatolites, Hebei, China. Acta Micropaleontol Sin 4:1–12

Part I

Environments

Cyanobacteria in Geothermal Habitats

3

David M. Ward, Richard W. Castenholz and Scott R. Miller

Contents

Summary

In the last decade advances in high-throughput DNA and RNA sequencing have driven more intensive surveys of cyanobacterial diversity in geothermal systems worldwide and the development of a deeper understanding of well-studied hot spring cyanobacterial communities. As a consequence, it is now possible to build, atop the long-term studies of these systems based on morphological, pure-culture and initial 16S rRNA observations, a more thorough understanding of the biogeographical and local distributions of cyanobacteria in these settings. Population genetics studies with increased molecular, spatial and temporal resolution have begun to define the ecological species populations of thermophilic cyanobacteria and to reveal the processes that drove their evolution and current ecology. Metagenomic studies have begun to reveal the functional gene repertoire of the predominant cyanobacteria and associated members of communities in which they reside and with whom they interact. Gene expression studies, including metatranscritomic studies, have begun to reveal patterns of *in situ* gene expression.

3.1 Introduction

Dramatic advances in high-throughput molecular approaches in microbial ecology have, in turn, dramatically improved understanding of thermophilic cyanobacteria and the communities they inhabit. The chapter contributed to the

D.M. Ward (✉)
Land Resources and Environmental Sciences Department, Montana State University, Bozeman, MT 59717, USA
e-mail: umbdw@montana.edu

R.W. Castenholz
Institute of Ecology and Evolutionary Biology, University of Oregon, Eugene, OR 97403-5289, USA
e-mail: rcasten@darkwing.uoregon.edu

S.R. Miller
Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA
e-mail: scott.miller@mso.umt.edu

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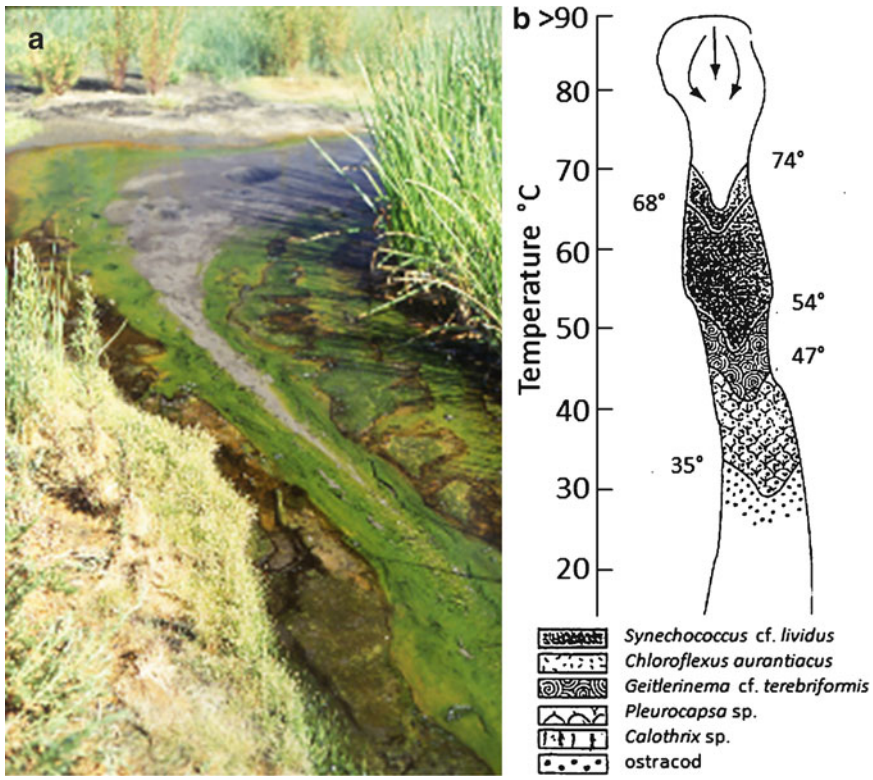


Fig. 3.1 Hunter's Hot Springs, Oregon: (**a**) Photograph and (**b**) schematic depicting the approximate downstream distribution of the predominant cyanobacteria, *Chloroflexus aurantiacus* and one grazing invertebrate present at the mat surface in the outflow of most springs in the Hunter's Hot Spring group

previous edition of this book (Ward and Castenholz 2000) emphasized how molecular methods developed in the 1990s had reshaped our knowledge of cyanobacteria in geothermal habitats. In that chapter, we compared the traditional view offered by microscopy and cultivation with the view emerging through 16S rRNA analyses. We also expanded the description of well-studied geothermal microbial communities initiated by Castenholz (1973a) with the description of Hunter's Hot Springs, OR (Fig. 3.1) by adding a description of work conducted at Octopus Spring in Yellowstone National Park (YNP). This new chapter emphasizes what has been learned in the past decade as 16S rRNA methods have been used to more extensively survey the cyanobacteria of geothermal habitats and, as genomics, metagenonomics, metatranscriptomics and metaproteomics have deepened understanding of cyanobacteria in well-studied model systems in Mushroom Spring (Fig. 3.2) and White Creek (Fig. 3.3) in YNP. As a result, we rely on the previous chapter (Ward and Castenholz 2000) for coverage of work completed prior to 2000. Many portions of that chapter have been omitted and reference will be made to figures and tables of that chapter when necessary. The cyanobacteria found in geothermal habitats, based on both phenotype and genotype are listed in Table 3.1.

3.2 Distribution of Thermophilic Cyanobacteria Based on Morphology and Cultivation

Differences in the composition of cyanobacterial populations among diverse hot springs can often be discerned by microscopic examination, based on distinctive morphological characteristics (Table 3.1 and Fig. 3.2; see also Fig. 1 of Ward and Castenholz (2000)), although true genotypic differences or similarities are not revealed by microscopy. Differences observed between springs a few meters or kilometers from each other are likely to be due to local variations in chemical composition, temperature or exposure to solar irradiance. Those differences observed among widely separated springs are more likely the consequence of geographic isolation and the limitations of dissemination. Morphology can also be assessed from culture isolates from the springs.

3.2.1 Geographic Distribution

Geothermal springs are located non-continuously and may be likened to islands. They are scattered on all continents except Antarctica (but steam vents occur on two Antarctic

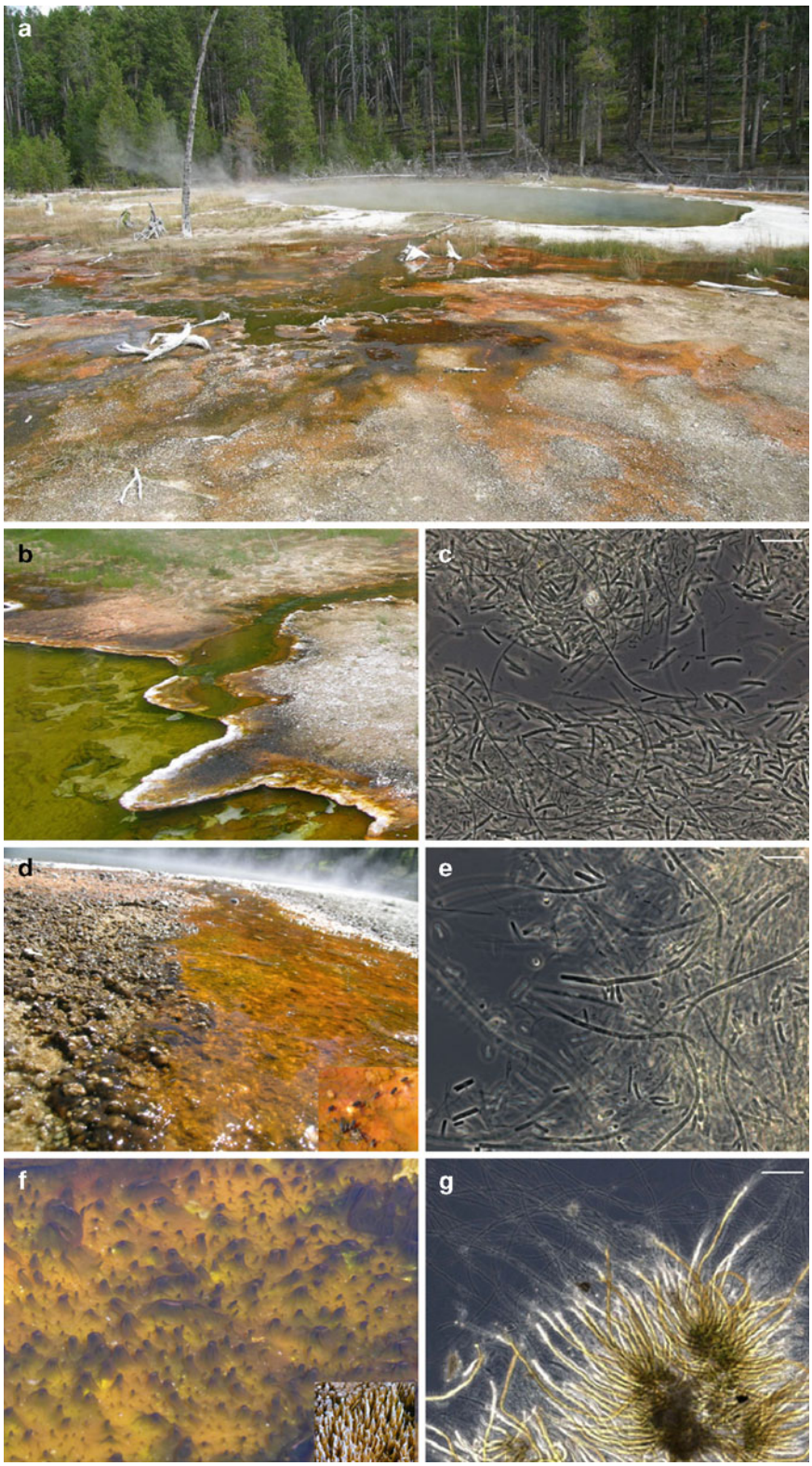


Fig. 3.2 Mushroom Spring, YNP and its microbial communities: (**a**) Landscape showing the *yellow-green mat* lining the source pool and downstream mats in foreground; (**b**) *Synechococcus* mats in source pool and effluent channel; (**c**) Photomicrograph of *Synechococcus* cells and filaments in *upper green mat* layer; (**d**) *Low-angle view* of downstream *Leptolyngbya* (*Phormidium*) (*orange mat* on *right*) and *Calothrix* (*brown tufts* on *left*) communities with source pool in background at *top* of photo; *inset* shows ephydrid flies on *Leptolyngbya* (*Phormidium*) mat; (**e**) Photomicrograph of *Leptolyngbya* (*Phormidium*) filaments together with *Synechococcus* cells and bacterial filaments from an *orange mat* like that shown in (**d**); (**f**) Conical structures in a *Leptolyngbya* (*Phormidium*) mat within a quiescent pool; *inset* shows silicified cones upon dehydration; (**g**) Photomicrograph of *Calothrix* filaments typical of those in the *brown mat* in (**d**). All photomicrographs are phase contrast images; scale bar is ~10 μm in (**c** and **e**) and ~100 μm in (**g**)

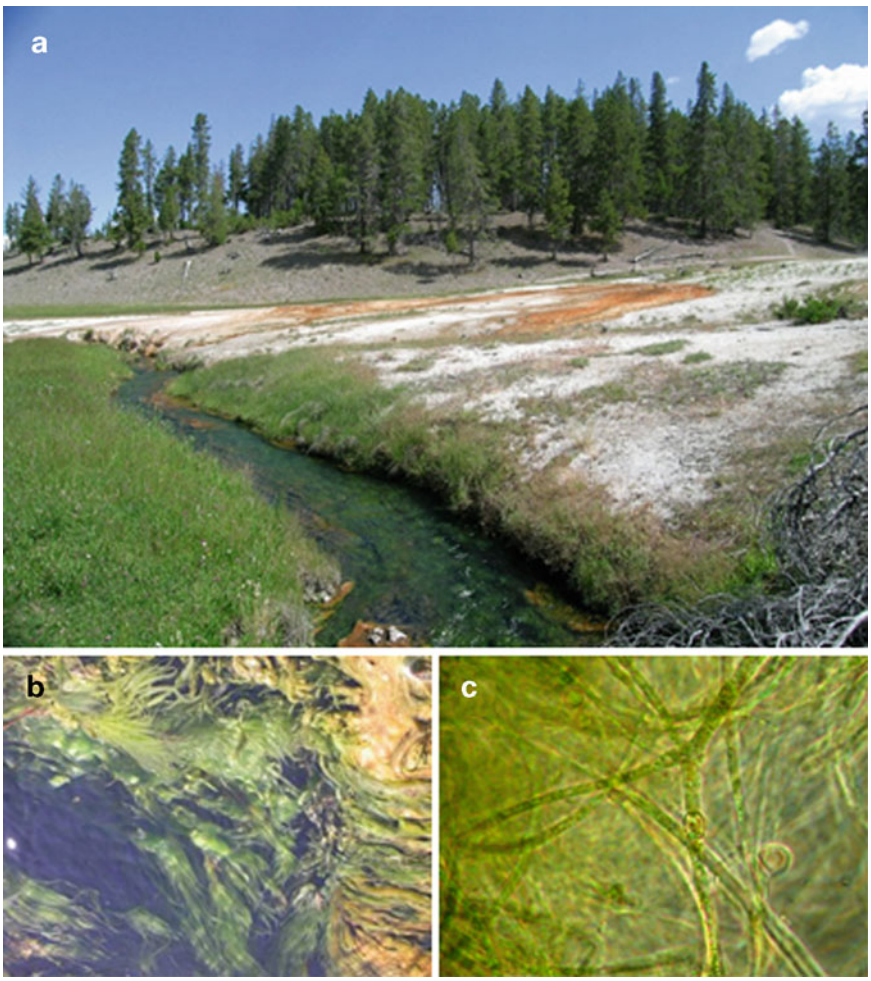


Fig. 3.3 White Creek, YNP: (**a**) *Landscape view* looking downstream with lower temperature influents from Octopus Spring and other nearby hot springs to the *right*; (**b**) Streamers containing *Fischerella laminsosus* are found below ~55°C; (**c**) Phase contrast micrograph of White Creek mat (~53°C). Note the true-branching pattern and heterocyst of *F. laminosus* near image *centre*

volcanoes) and on many island groups. Hot springs are mainly associated with current or recent volcanic activity, but also with active faulting where surface waters sink deeper, are heated and then resurface. Often there are great distances separating hot spring clusters. It is not unreasonable to believe that there should be endemic species of thermophiles, restricted to certain hot spring groups as a result of geographic isolation and evolutionary divergence of the microbes. This concept should not be surprising, since dispersal of obligate thermophiles from rare and distant point sources is certainly a limiting factor for some taxa, and the time between successful long-distance disseminations may be enough to allow speciation to take place.

A striking anomaly suggesting geographically restricted distributions of thermophilic cyanobacteria is that all forms of thermophilic *Synechococcus* appear to be absent from Icelandic hot springs (although numerous springs exist there that appear chemically suitable) (Castenholz 1978) (Table 3.1). Thermophilic *Synechococcus* (with slightly different morphologies) occur in New Zealand and European springs, but include only forms that grow up to temperature limits of about 62°C and 58°C, respectively (Castenholz 1969, 1976, 1978, 1996; Ward and Castenholz 2000). Forms of *Synechococcus* that grow in nature up to limits of 73–74°C in the western contiguous United States and south into Central and South America, are absent in the geographic regions mentioned above, although they extend into eastern Asia (Thailand (Sompong et al. 2008; Castenholz, unpublished data) and China (Yun 1986)). In contrast, some thermophilic cyanobacteria, such as *Fischerella (Mastigocladus) laminosus* morphotypes and the high temperature forms (HTF) of "*Chlorogloeopsis*" appear to be cosmopolitan in distribution (Castenholz 1996). Latitude and daylength do not appear to be very important in the distribution of thermophilic cyanobacteria, since many morphotypes similar to those of lower latitude springs occur in the hot springs of the east coast of Greenland at 70–71°N. These springs experience complete darkness in winter and constant daylight in summer (Roeselers et al. 2007).

Table 3.1 Cyanobacteria found in geothermal habitats, arranged top to bottom in order of their occurrence along a thermal gradient, their genetic affiliation, representative cultivars and distribution relative to biogeography and environmental parameters

Morphological genus species	16S rRNA genotype	Representative strains	Biogeographical distribution based on morphotype (M), environmental sequence (E) or sequenced laboratory strain (L)															Max °C	pH	Sulfide tolerance	N_2 fixation
			NA	LA	NZ	JA	PH	TH	TI	IC	EU	AU	AF	GR	RU	CH	ME				
Synechococcus cf. *lividus* (HTF)			M	M				M								M		73–74	>6	Low	
	A″		E															72			Yes
	A′		E															72			Yes
		Miller Group IV	L																		
	A		E,L															67			
		Miller Group III	L																		
		Allewalt JA-3-3Ab																			Yes
Synechococcus cf. *lividus*			M	M	M	M	M	M	M		M		M			M		58–66	>pH 5	Low (varies)	Varies
	B′	Allewalt JA-2-3B′a(2-13)	E,L															63			Yes
		Miller Group II	L																		
	B	Allewalt TS-15	E,L															57			
	T1-like		E	E				E,L	E						L		E				
	C1-like		E,L			E,L	E	E,L	E								E				
	C9-like		E,L		E	E		E									E				
Cyanothece (*Synechococcus*) cf. *minervae*			M,E,L	M					E									62	>6	Low (?)	No
"*Chlorogloeopsis* HTF"			M	M	M					M	M							64	>4.5	Low (~0.15 mM)	Yes
Leptolyngbya (*Phormidium*) spp. (cf. *P. laminosum*)			E,L				E				L						E	~62		Mod. (?)	No
	OS Type I		E																		
Fischerella (*Mastigocladus*) cf. *laminosus*			M, E,L	L	L	E,L	E	E		L	L	E					E,L	58	>5	Mod. (~0.25 mM)	Yes
Leptolyngbya (*Oscillatoria*) cf. *amphigranulata*					M, E,L	E	E	E				E		L	L			~56	>6.5	High (~3 mM)	No
Geitlerinema (*Oscillatoria*) cf. *terebriformis*			M,L	M												M	L	55	>6	High (~1 mM)	No
Spirulina cf. *labyrinthiformis*			M															51	>6	Mod (~0.1 mM)	No
Calothrix spp.			M															~53-55	8-8.5	Low (?)	Yes
Pleurocapsa spp.			M															57			Yes

The first three columns are hierarchical in the sense that each morphotype is associated with genotypes in following rows and each genotype is associated with strains in following rows. For instance, the morphotype *Synechococcus* cf. *lividus* (HTF) is grouped with three A-like genotypes; one strain representing genotype A′ and two strains representing genotype A and have been cultivated

The genomes of strains JA-2-3Ab, a close relative of strain JA-3-3Ab (see Allelwalt et al. 2006), and JA-2-3B′a(2-13) have been obtained (Bhaya et al. 2007)

NA North America, *LA* Latin America, *NZ* New Zealand, *JA* Japan, *PH* Phillipines, *TH* Thailand, *TI* Tibet, *IC* Iceland, *EU* Europe, *AU* Australia, *AF* Africa, *GR* Greenland, *RU* Russia, *CH* China, *ME* Middle East

(?) unknown, but inferred from ecological setting

3.2.2 Distributions Determined by Temperature and Chemistry

Within a local biogeographical region, temperature and pH, in combination with availability of combined nitrogen, phosphorus and other nutrients and/or concentration of free sulphide (i.e. H_2S, HS^-, S^{2-}) determines the distribution of thermophilic cyanobacteria (Table 3.1).

3.2.2.1 Temperature

Table 3.1 is organized to show the approximate distributions of thermophilic cyanobacteria relative to temperature (high to low listed top to bottom). The most notable differences in a biogeographical sense are the limited distributions of high-temperature forms. Since all of the listed taxa are not present in a particular system, we will rely on Sect. 3.2.3 to provide further insight into distribution related to temperature in well-studied systems.

3.2.2.2 pH

In hot springs worldwide, cyanobacteria are not observed below a pH of about 4.0, and their diversity seems quite limited below pH 6 (Brock 1973). In Yellowstone (Clearwater Springs and Norris Geyser Basin) *Synechococcus* spp. or varieties occur in hot springs with pH values as low as ~5.2, and "HTF *Chlorogloeopsis*" populations occur at pH levels as low as ~4.5 (see Ward and Castenholz 2000). The *Synechococcus* (clone Y-7C-s) isolated from a pH 5.5 spring in the Clearwater group grew at maximal rates only at pH levels above pH 7 and thus appeared acidotolerant not acidophilic (Kallas and Castenholz 1982a, b).

3.2.2.3 Nitrogen and Phosphorus Availability

When the outflows of neutral to alkaline, non-sulphidic hot springs containing combined nitrogen have cooled to 73–74°C there is the likelihood (at least in the above mentioned geographic regions) that a high temperature form (HTF) of *Synechococcus* will be present as a biofilm or mat, which may, in turn, influence the chemistry downstream where other species of cyanobacteria enter the thermal gradient. For example, the combined nitrogen (usually as NH_4^+) in the spring source may be largely removed by *Synechococcus*. The *Synechococcus* ecotypes or species may then be succeeded downstream (at least below ~58°C) by heterocystous, N_2-fixing, cyanobacteria, most commonly *Fischerella (Mastigocladus) laminosus* (e.g. White Creek, Lower Geyser Basin, YNP) but elsewhere by *Calothrix* spp. below ~53–55°C (unpublished observations). Nitrogen fixation has been measured at approximately 60°C in hot spring *Synechococcus* (Steunou et al. 2006, 2008), and at lower temperatures where heterocystous *Fischerella* and *Calothrix* occur (Stewart 1970; Wickstrom 1980). In contrast, a spring may be rich enough in combined nitrogen to favour *Fisherella* without heterocysts (Miller et al. 2006). In other cases, combined nitrogen may be very low at the source, even in high-temperature springs, and heterocystous cyanobacteria may constitute the upper-temperature species at the upper limit for growth (e.g. "HTF *Chlorogloeopsis*" at 64°C in New Zealand). Possible distribution differences based on phosphorous availability have been suggested by comparison of genomes of *Synechococcus* strains representative of populations at different positions along the flow path (Sect. 3.4.1.1).

3.2.2.4 Sulphide Tolerance and Utilization

Many neutral to alkaline geothermal springs contain primary soluble sulphide in the source water. Sulphide is an effective inhibitor of photosynthesis and possibly other physiological processes in the majority of cyanobacteria, but may be used as a photosynthetic electron donor in some sulphide-tolerant species (Cohen et al. 1986; Castenholz and Utkilen 1984). Present evidence indicates that no thermophilic cyanobacteria with the capacity to grow above 56°C are capable of growing in waters with more than ~10 μM sulphide (Castenholz 1976, 1977; Garcia-Pichel and Castenholz 1990). In sulphide-rich springs of New Zealand the upper-temperature, sulphide-tolerant and sulphide-utilizing cyanobacterium is a *Leptolyngbya* ("*Oscillatoria*") *amphigranulata* morphotype (Castenholz 1976). In some hot springs of the upper Mammoth Terraces, Yellowstone Park, where source temperatures are ~52°C or below, a sulphide-utilizing *Spirulina labyrinthiformis* morphotype (with an upper temperature of 51–52°C) predominates near the sulphide-rich source (Castenholz 1977). In higher temperature springs, with similar sulphide concentrations at the source, waters usually lose all detectable sulphide by the point where the outflow reaches 52°C and are dominated by less sulphide-tolerant species at that temperature and below. Non-photosynthetic sulphide-oxidizing bacteria (e.g. *Sulfurihydrogenibium*) predominate in zones below 75–77°C sources in several sulfidic hot springs in the upper terraces of Mammoth Hot Springs, YNP (e.g. Inskeep et al. 2010) and are likely to be mainly responsible for sulphide removal in the upper temperature zone. Icelandic and Yellowstone springs with primary sulphide often have mats of photoautotrophic filamentous anoxygenic bacteria (FAPs; e.g. *Chloroflexus, Roseiflexus*) at temperatures of ~66°C down to a temperature where surface-water sulphide disappears (<~60°C) (Castenholz 1973b; Giovannoni et al. 1987; Klatt et al. 2012b). In a few Icelandic mats, sulphide-oxidizing FAPs on the mat surface remove sulphide, permitting sulphide-sensitive cyanobacteria to grow beneath them in the lower part of the photic zone (Jørgensen and Nelson 1988).

3.2.2.5 Salinity

Although saline hot springs are rare, those that arise as hot altered seawater on the Reykjanes peninsula of southwestern Iceland (mid-Atlantic Ridge terrestrial outflow)

harbor cyanobacteria at temperatures near 40°C. However, isolates of *Leptolyngbya* from this water (the Blue Lagoon) grew well at three times the salinity of seawater and at a temperature of 55°C, which was also an unexpected characteristic of some *Leptolyngbya* isolates from endolithic habitats in ancient Yellowstone travertine (Banerjee et al. 2009).

3.2.3 Well-Studied Mat Systems

Brief consideration of cyanobacterial diversity based only on morphology and cultivation is provided in this section. More detailed descriptions based on molecular sequence data are presented in Sects. 3.3 and 3.4.

3.2.3.1 Hunter's Hot Springs, Oregon

These privately owned springs, 3.5 km north of Lakeview, Oregon (elev. 1,470 m), have been studied periodically by Castenholz and students since the early 1960s (Fig. 3.1a). They consist of a series of springs initiating from an active deep faulting system, many issuing at temperatures of about 90°C with a pH of 8.0–8.4. The major ions are sodium (~8.6 mM), chloride (~3.2 mM), silicate (~1.2 mM), bicarbonate (~1.7 mM) and sulphate (~2.8 mM) (specific conductance of ~1.1 mS). The chemistry and microbiota of Hunter's Hot Springs is characteristic of many hot springs of the Great Basin (C.E.Wingard and R.W.Castenholz, unpublished data; Mariner et al. 1974).

Besides identifying the conspicuous cyanobacteria, the objective of early studies was to explain the abrupt upper and lower temperature boundaries of distinctive phototrophic populations in a continuous and linear temperature gradient. These distributions are illustrated in Fig. 3.1b and are described in Castenholz (1969, 1973a) and Wickstrom and Castenholz (1978, 1985). Briefly, the upper temperature limit for cyanobacteria, and for global photosynthesis, is almost certainly 73–74°C and this boundary is easily seen when the temperature remains relatively constant at a particular point in the outlet. This greenish-yellow mat, composed of a cyanobacterial form-species identifiable morphologically as *Synechococcus lividus* Copeland occurs as the top cover of the mat in most of the Hunter's Spring outflows to about 54–55°C where it is abruptly replaced by a dark reddish-brown cover of *Geitlerinema* (*Oscillatoria*) *terebriformis*, a distinctive form identified by morphology, physiology, ecology, and recently by 16S rDNA sequences (Castenholz 1978, 1996, and T.B. Norris, unpublished sequence data). The uniform green cover of *Synechococcus*, however, was found to consist of at least four stable thermotypes that appear microscopically similar (Peary and Castenholz 1964; Miller and Castenholz 2000) (Fig. 3.4a, c). In culture, the most thermotolerant clone grew at a maximum rate at 63–68°C, but grew up to a temperature of 72°C and not below 55°C (Meeks and Castenholz 1971; Miller and Castenholz 2000). In culture, with continuous illumination, the maximum growth rate of this strain was slightly <1 doubling/24 h, considerably slower than the lower temperature strains, which were able to grow optimally at temperatures well below 55°C, but which could not grow at the high temperature of the most thermotolerant strain (Miller and Castenholz 2000). The doubling rates in Peary and Castenholz (1964) were considerably greater than those in Miller and Castenholz (2000) (compare Fig. 3.4a, b), possibly due to differences culture conditions with higher light intensity in Peary and Castenholz (1964) or in the genotypes of the strains.

G. terebriformis has an upper growth temperature limit of 54.5°C in culture (and in nature) and grows at a maximal rate close to this upper limit (Castenholz 1968, 1973a). Since it is highly motile (by gliding), the upper edge of the mat adjusts its position to its upper temperature limit. Since the mat is generally thick enough to absorb over 95% of visible radiation, this cover sets the lower boundary of the otherwise extensive *Synechococcus* mat, which subsequently becomes light-limited. Although all the clonal cultures of *G. terebriformis* have shown growth rates of over 2 doublings/24 h below 48°C (Fig. 3.4a), in most springs of E. Oregon and N. Nevada, the *Geitlerinema* mat ends abruptly at about 47–48°. This is a result of dense, voracious populations of the thermophilic and "herbivorous" ostracod *Thermopsis thermophila* (ex *Potamocypris* sp.) that are nearly ubiquitously distributed in the same geographic region (Castenholz 1973a; Wickstrom and Castenholz 1985; Külköylüoglu et al. 2003).

Since this species of ostracod can survive and reproduce at temperatures as high as 48°C and possibly higher (Wickstrom and Castenholz 1973; Külköylüoglu et al. 2003), it ingests the delicate trichomes of *Oscillatoria* and the soft undermat of *Chloroflexus* at a rapid rate. However, the cyanobacterial population below this temperature is composed primarily of the highly grazer-resistant *Pleurocapsa* sp. and *Calothrix* sp. The tapered filaments of *Calothrix* are embedded within the nearly amorphous mass of *Pleurocapsa* cells (Wickstrom and Castenholz 1978). The ostracods appear to graze primarily on the exposed lawn of the tips of *Calothrix* filaments which continue to grow between the *Pleurocapsa* cells below 48°C and also on masses of *G. terebriformis* which are washed downstream (Wickstrom and Castenholz 1985). Combined nitrogen may be limiting in the flowing water over this portion of the mat, since both the *Calothrix* and *Pleurocapsa* are capable of nitrogen fixation. By comparing the drainways of similar springs with and without ostracods it has become obvious that the *Pleurocapsa/Calothrix* community is not only quite grazer-resistant, but is actually grazer-dependent. It does not occur when the ostracods are absent. In such spring outflows the *Geitlerinema* (*Oscillatoria*) *terebriformis* cover extends downstream to about 35°C.

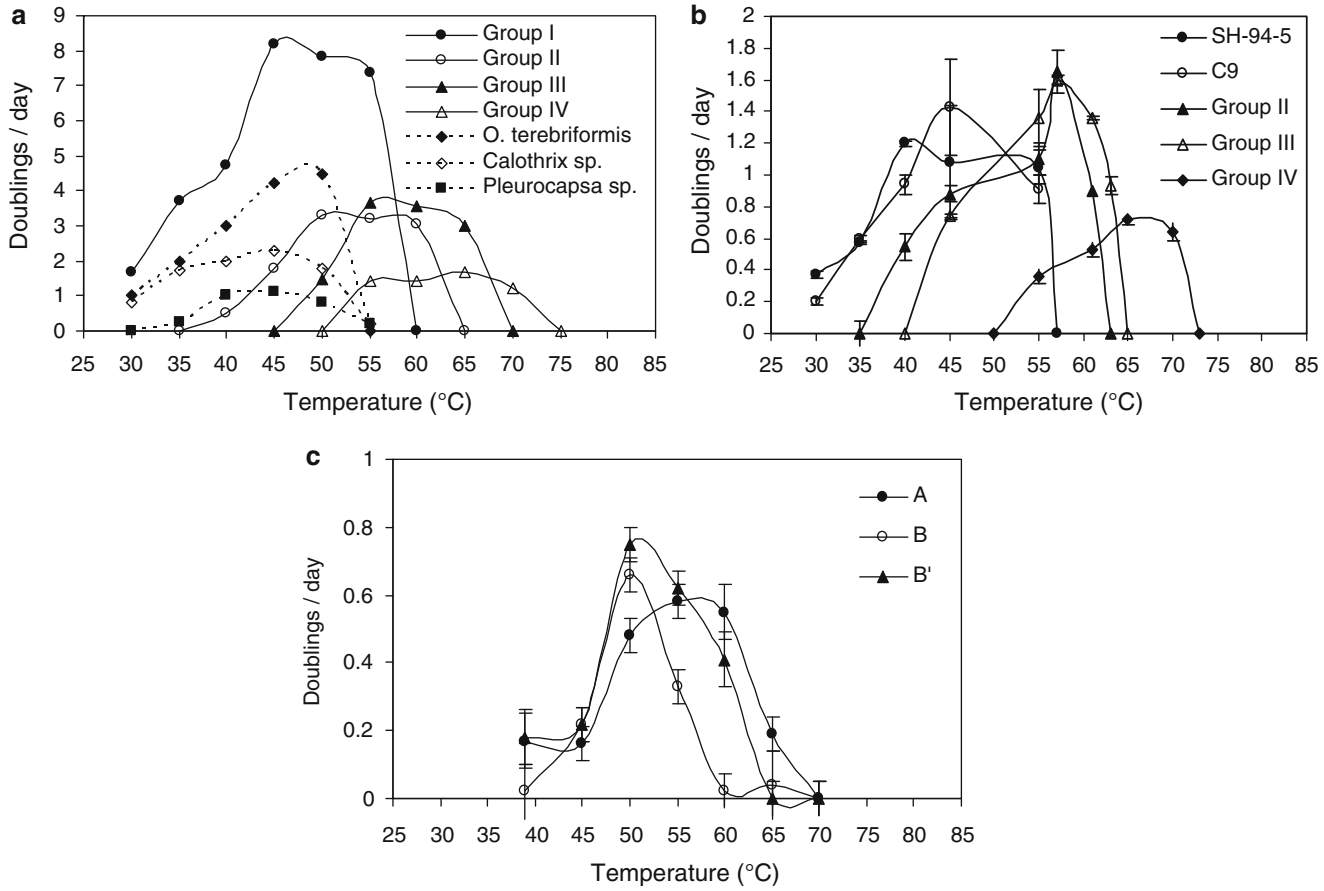


Fig. 3.4 Effect of temperature on growth rates of cyanobacterial isolates from (a, b) Hunter's Hot Springs, OR: (**a**) from Peary and Castenholz (1964); (**b**) from Miller and Castenholz (2000); (**c**) Octopus Spring, YNP: Allewalt et al. (2006). Except as noted in (**a**) all strains are *Synechococcus* thermotypes

3.2.3.2 Mushroom Spring and Octopus Spring, Yellowstone National Park

Mushroom Spring and Octopus Spring, located in the Lower Geyser Basin, YNP (Octopus Spring is in the White Creek drainage), have been studied extensively by T.D. Brock, Castenholz, Miller, Ward, their students and many others since the late 1960s. These are alkaline springs (pH ~8.3) of volcanic origin. Like Hunter's Hot Spring, the major ions are sodium (~13 mM) chloride (~8.2 mM), bicarbonate (~4.8 mM) and silicate (~1.8 mM). Unlike Hunter's Hot Spring, these springs have very low sulfate content (~0.3 mM) (Brock 1978; Papke et al. 2003). Until the mid-1990s, work was primarily conducted at Octopus Spring, but a major disturbance event (likely a hailstorm) that temporarily destroyed the Octopus Spring mat precipitated a shift to focusing primarily on nearby Mushroom Spring in the years since. Here we describe work done mainly at Mushroom Spring since that time (see Ward and Castenholz (2000) for a comparable description of the Octopus Spring landscape). These two springs are highly similar in their microbiota (compare Ward et al. 1998 and 2006). One interesting difference between these two habitats is the cooler source pool (see below); another is the cycling of temperature in Octopus Spring, which exposes sites in the effluent channel to shifts in temperature of ~10°C every 5–10 min (Miller et al. 1998). This may have interesting consequences for cyanobacterial diversity and its role in stabilizing oxygenic photosynthesis (see Figure 7 in Ward and Castenholz 2000).

A landscape view of Mushroom Spring and its cyanobacterial features is shown in Fig. 3.2a. As the source pool temperature (~70°C) is cooler than the upper temperature limit for cyanobacteria (~73–74°C), it is lined with a several millimetre thick translucent mat, which is more yellow-green at the surface and dark-green a few millimeters below the surface (Fig. 3.2b). This mat is comprised of *Synechococcus* cells resembling *S.* cf. *lividus* (Fig. 3.2c). As the source water flows into the effluent channels and cools downstream, these yellow-green and dark-green layers compress from a several millimeter thick translucent mat at ~68°C to a <1 mm-thick upper green layer at ~65°C and below (Figs. 3.2b and 3.5a). *Synechococcus* thermotypes have also been found in these systems (see Sects. 3.3.2 and 3.4.1.1). At temperatures

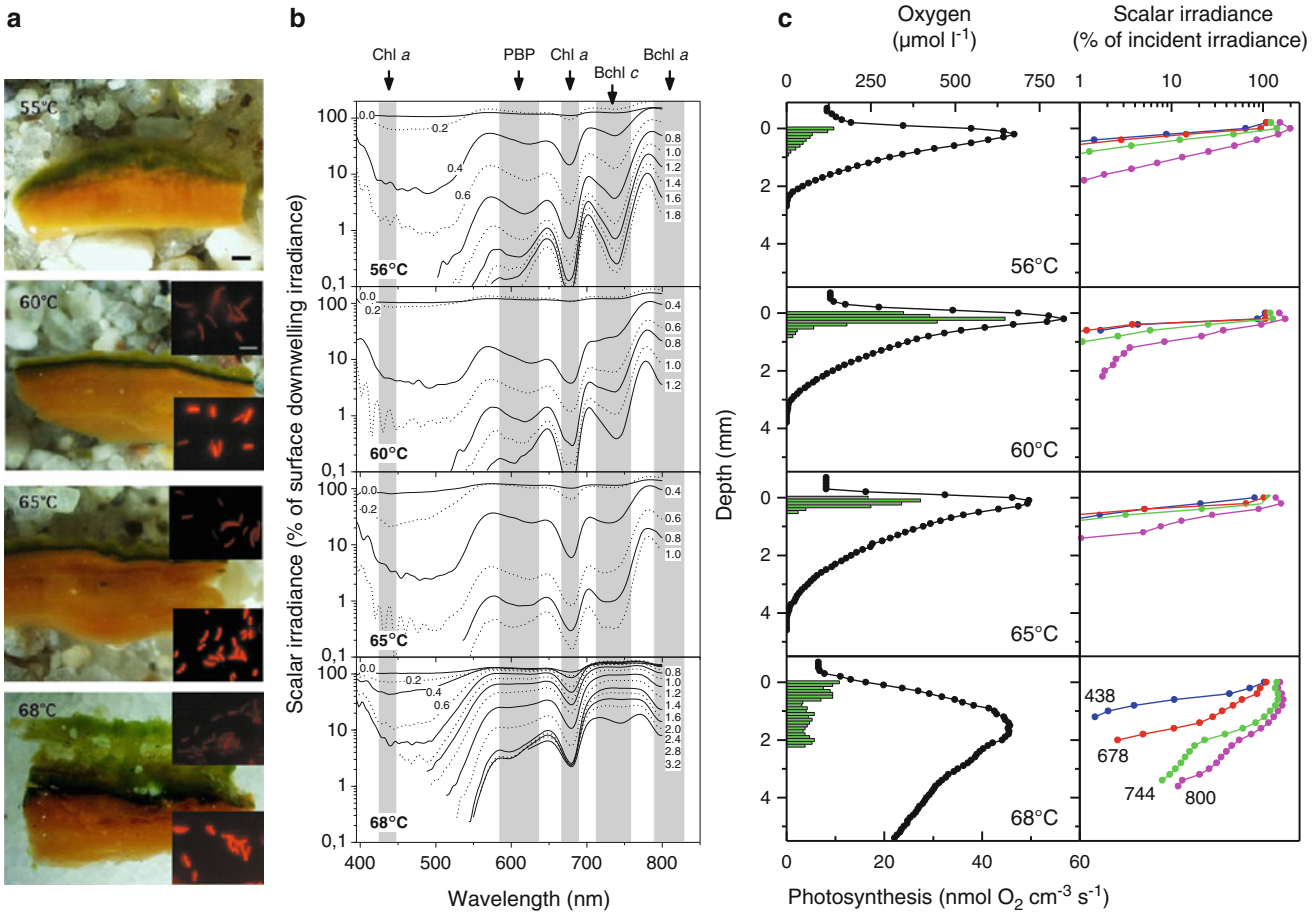


Fig. 3.5 Vertical aspect of *Synechococcus* mats in Mushroom Spring, YNP: (**a**) Cross sections of mat samples collected at 55°C, 60°C, 65°C and 68°C and autofluorescence photomicrographs of *Synechococcus* cells in the surface and subsurface parts of the top-green mat layer. *Scale bar* indicates 10 μm. (**b**) Vertical profiles of scalar irradiance of light of different wavelengths with depth (indicated in mm next to *lines*; *PBP* phycobiliprotein). (**c**) Vertical microsensor profiles of oxygen (*black line* and *points*), oxygenic photosynthesis (*green bars*) and penetration of light of specific wavelengths (From Ward et al. 2006)

between ~68°C and ~50°C, the *Synechococcus* cells in the upper yellow-green layer autofluorescence more dimly than those in the dark-green underlayer (Fig. 3.5a). Orange undermat layers are comprised largely of FAPs (e.g. *Chloroflexus* spp. and *Roseiflexus* spp.), as judged by fluorescence *in situ* hybridization with 16S rRNA probes (Nübel et al. 2002). These two types of FAPs are about equally abundant at 68°C, but *Roseiflexus* spp. predominate at lower temperatures. Overall mat thickness increases to up to several centimeters as temperature decreases along the effluent channel, though mat formation and decomposition are probably maximized in the upper few millimeters (Brock 1978; Ward et al. 1987).

Below approximately 58°C *Leptolyngbya* (*Phormidium*) spp. may be found together with *Synechococcus* spp., where they are especially obvious in reticulate features (Fig. 3.2d, e), streamers (in strong flow) and raised columns or pinnacles (in quiescent pools; Fig. 3.2f). *Leptolyngbya* (*Phormidium*)-dominated mats are usually orange in summer (Norris et al. 2002). The laminated mats and conical structures have been extensively studied as analogs of their fossil equivalents, planar stromatolites and the pinnacled Conophyton forms (see Walter et al. 1972; Brock 1978; Awramik and Vanyo 1986; Vanyo et al. 1986). Below approximately 43°C the larvae and adults of ephydrid flies graze upon the cyanobacterial mat (Fig. 3.2d inset). The thermophilic ostracod described in Sect. 3.2.3.1 occurs mainly in hot springs of the Great Basin region (e.g. Hunter's Hot Springs), and has not been found in any YNP springs. In the cooler waters further downstream (~40°C-ambient), N_2-fixing *Calothrix* forms brownish scytonemin-containing mats adhering to the siliceous substratum both in the flow and on the moist edges of the effluent (Fig. 3.2d, g).

3.2.3.3 White Creek

White Creek is a tributary of the Firehole River which drains many thermal features of the Lower Geyser Basin of Yellowstone National Park, including Octopus Spring and

Great Fountain Geyser (Fig. 3.3a). Its chemistry is similar to the primary hot springs it drains with respect to pH (~8.2), silicate (~4.2 mM) and sulfate (~–0.1 to 0.2 mM), but it has lower concentrations of sodium (~4.3 mM) and chloride (~1.4 mM), presumably due to dilution by surface stream water (Miller, unpublished data). White Creek, as a high volume and velocity stream, is characterized by a much shallower thermal gradient (2°C per 100 m) than the primary thermal features (Miller et al. 2009a). Consequently, cyanobacterial mat communities are distributed along an approximately 1.5 km stretch of the channel with mean annual temperature ranging between approximately 72°C and 39°C (including a large hot spring that serves as one of the primary upstream sources of geothermally heated water).

At temperatures greater than about 55°C, laminated mats of *Synechococcus* and FAPs similar to those shown in Fig. 3.2 predominate. Between ~55°C and 64°C, large cells of the heterocyst-forming *Chlorogloeopsis* are also occasionally evident. At lower temperatures, between ~39°C and 55°C, streamer mats composed of *Fischerella (Mastigocladus) laminosus*, other cyanobacteria and FAPs are observed (Fig. 3.3b, c). Few sites in YNP harbour large amounts of *F. laminosus* biomass, though this cyanobacterium has been obtained from many locations in enrichment cultures, and the generally low concentrations of combined N that are measured in this region of White Creek (Miller et al. 2006, 2009b, unpublished data) likely contribute to its success. Intercalary heterocysts are evident in *F. laminosus* trichomes, and mats actively fix N_2 as assayed by acetylene reduction (Stewart 1970; Miller et al. 2006). Members of the *F. laminosus* population have diverged in thermal performance (i.e. different thermotypes), and distribution of this ecological variation is tightly associated with environmental temperature (Sect. 3.4.2; Miller et al. 2009a). Particularly near the upstream population boundary, trichomes of *F. laminsosus* are tightly associated with adhered *Synechococcus*, whereas at downsteam locations other cyanobacteria are more evident.

3.3 Distribution of 16S rRNA–Defined Taxa

The contemporary distributions of cyanobacteria in geothermal habitats are potentially shaped by various factors, including dispersal limitations that result in biogeographic patterns and environmental heterogeneity (i.e., habitat sorting). We begin with a global perspective before appraising the contribution of parameters related to effluent flow away from source pools (e.g., temperature) to the realized niches (i.e. actual *in situ* distributions, as opposed to the fundamental or potential niches defined by laboratory studies) of thermophilic cyanobacteria in local settings.

3.3.1 Biogeographic Distribution

A current focus of microbial ecology has been on the issue of how microorganisms are distributed at a variety of geographic scales (reviewed by Martiny et al. 2006). Determining the spatial structure of microbial diversity is a prerequisite for developing an understanding of the processes that shape distribution patterns. A question of particular interest is whether dispersal barriers to migratory gene flow exist for microorganisms, i.e., whether microbial populations are geographically isolated (Finlay 2002; Papke et al. 2003; Whitaker et al. 2003). Genetic divergence of populations at neutral loci is expected to increase with physical distance in the absence of significant migration (Wright 1943), because local selective sweeps (Sect. 3.4.1.2) and genetic drift remove variation within, but not between, populations. Although it has long been recognized from morphological surveys of microbial mat communities from geothermal habitats that certain cyanobacteria exhibit restricted geographic ranges (Sect. 3.2.1; Castenholz 1996; Ward and Castenholz 2000), advances in molecular biological approaches for investigating microbial diversity *in situ* have greatly enhanced our appreciation of this biogeographic structure (Fig. 3.6). Papke et al. (2003) provided the first study of hot spring cyanobacterial 16S rRNA diversity at global and regional scales. For environmental samples from North America, Japan, New Zealand and Italy, the authors (i) confirmed that taxa vary in their breadth of distribution, (ii) reported that patterns of taxon distribution could not be explained by spring geochemistry, and (iii) concluded that allopatric divergence of geographically isolated populations generally plays a key role in the origins and maintenance of diversity. Surveys of molecular diversity from other geothermal regions (as well as numerous unpublished environmental sequence data in public databases) have provided a rich comparative data set that generally corroborates the conclusions of Papke et al. (2003). Included in these surveys were collections from Australia (Anitori et al. 2002), Greenland (Roeselers et al. 2007), Jordan (Ionescu et al. 2010), The Philippines (Lacap et al. 2007), Thailand (Jing et al. 2005; Portillo et al. 2009; Sompong et al. 2008; Lau et al. 2009), and Tibet (Lau et al. 2006, 2008, 2009). Below, we summarize the distribution patterns of representative taxa.

Many thermophilic cyanobacteria exhibit severely restricted geographic distributions (Table 3.1, Fig. 3.6). Perhaps the most notable example is the *Synechococcus* A/B clade (defined in Sect. 3.4.1). Definitive evidence for its presence, based on the recovery of environmental sequences and/or sequenced laboratory isolates, currently exists only from North American hot springs (e.g. Ward et al. 1998; Miller and Castenholz 2000; Papke et al. 2003; Allewalt et al. 2006). There is morphological evidence for the presence of high-temperature forms of *Synechococcus* in hot springs of China

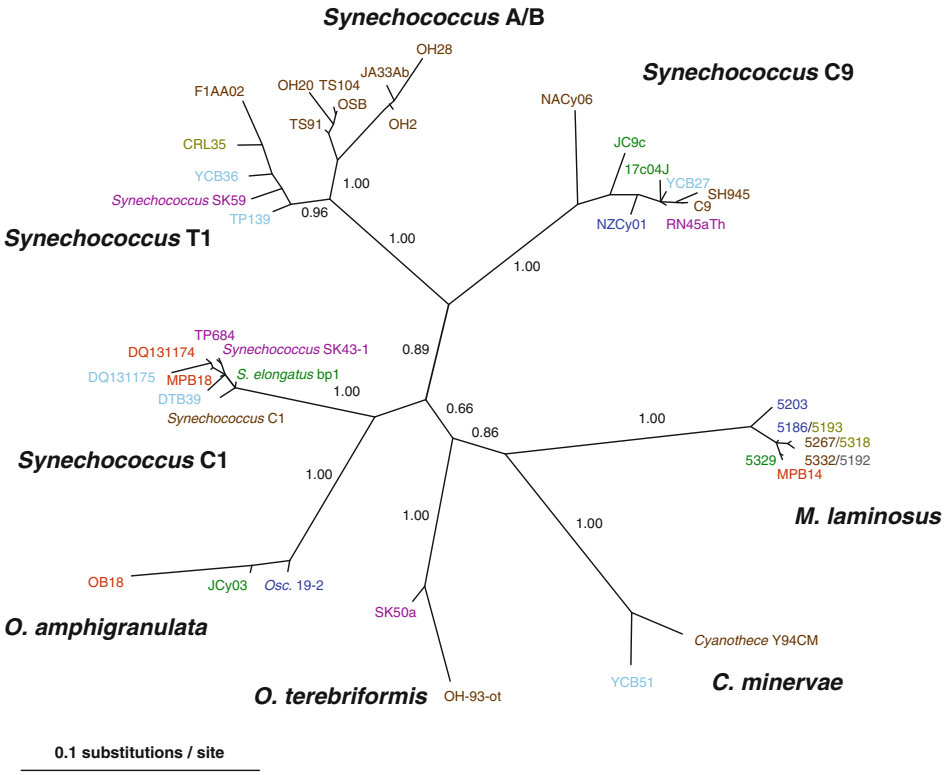


Fig. 3.6 Bayesian phylogeny (Huelsenbeck et al. 2001) of the 16S rRNA gene emphasizing the geographic distributions of representative thermophilic cyanobacteria. Sequences were obtained from: North America (*brown*); Japan (*green*); the Philippines (*orange*); Thailand (*purple*); Tibet (*light blue*); New Zealand (*dark blue*); and Central/South America (*olive*). Two independent Metropolis-coupled MCMC chains of 1,000,000 generations each were run in Mr. Bayes according to a GTR+G+I model of sequence evolution. Chain convergence was assessed by the average standard deviation of split frequencies. The consensus diagram shown was produced from a sample of 1,800 trees following discard of 10% of sampled trees as burn-in. Clade credibility values at nodes represent the posterior probability that the monophyly of a clade is supported by the data. For clarity, these values have been removed within individual clades

(Yun 1986), Thailand (Sompong et al. 2008), Central and South America and possibly Africa (Castenholz 1996), but the genotypes of these organisms are not yet known. Interestingly, molecular evidence has been obtained from several studies for a sister clade of the A/B clade (described as "Lineage T1" in Lau et al. 2009) that exhibits a far wider geographic distribution (Fig. 3.6). Environmental sequences of this group have been recovered from microbial mat communities from Tibet (Lau et al. 2006, 2008, 2009; Yim et al. 2006), Thailand (Jing et al. 2005; Sompong et al. 2008), Jordan (Ionescu et al. 2010), Costa Rica (unpublished GenBank sequence accession no. EF545646) and YNP. The YNP T1-like sequences are from lithified coniform structures in Black Sand Pool (see inset to Fig. 3.2f) (Lau et al. 2005), mesothermic microbial mat from the margins of an unidentified hot spring pool (unpublished GenBank sequence accession no. FJ885003) and from 38°C mat at Rabbit Creek, where it appears to be rare (an estimated ~0.5% of the community; Miller et al. 2009a). In addition, representatives of the T1 group have been isolated in laboratory culture from a 36°C enrichment of material collected from the Kotel'nikovskii hot spring of the Baikal rift (*Synechococcus* sp. 0431; Sorokovikova et al. 2008) as well as from San Kamphaeng hot spring in Thailand (Sompong et al. 2008). Although environmental data were not reported for many of the sites from which these sequences and strains were derived, the trend appears that members of the T1 group are found primarily at lower temperatures than are members of the A/B group. If this is the case, then an intriguing possibility is that tolerance of lower temperatures than in the A/B group might help to explain the broader distribution and apparently greater dispersal capabilities of the T1 group. It is also of interest whether members of the T1 group occupy higher temperature niches in regions where representatives of the A/B clade are absent.

Another example of a thermophilic cyanobacterium with an apparently very restricted distribution is *Cyanothece* (*Synechococcus*) cf. *minervae* (Table 3.1). This phycoerythrin-producing cyanobacterium is obvious microscopically in YNP collections, most notably from below about 62°C at Mammoth Hot Springs (e.g. Minerva Terrace, from which its name is derived) and from Chocolate Pots Spring, and it has

been isolated in laboratory culture from White Creek, from a hot spring near Lone Star Geyser and from South Harney Hot Springs in Oregon (Miller, unpublished data). With respect to evidence from environmental sequences, the only other location from which it has been recovered is from near Yibbug Caka, Tibet (64°C; Lau et al. 2009).

A taxon with an unusual distribution is *Geitlerinema* (*Oscillatoria*) cf. *terebriformis* (Table 3.1; Fig. 3.6). Molecular evidence for thermophilic members of this taxon comes from North America (Hunter's Hot Springs, OR, northern California, and Nevada, Idaho, western Montana, Alum Rock Park, CA), Saudi Arabia and Thailand (Sompong et al. 2008). Isolates from the western USA and Saudi Arabia have essentially identical 16S rDNA sequences (T.B. Noriis, unpublished data). However, close relatives of *G.* cf. *terebriformis* (>97% 16S rRNA sequence identity) have been found in a variety of freshwater locations (e.g. Lake Alexandrina, Australia, and a limestone sinkhole in Mexico). This suggests the possibility of a relatively recent evolutionary origin of thermophily in the group. Thermophilic, sulphide-tolerant *Leptolyngbya* (*Oscillatoria*) cf. *amphigranulata* is apparently restricted to New Zealand (Garcia-Pichel and Castenholz 1990), Japan (Papke et al. 2003) and the Philippines (Lacap et al. 2007) (Fig. 3.6).

By contrast, many taxa appear to be more widely distributed: These include the *Synechococcus* C1 and C9 groups, and the heterocyst-forming *Fischerella* (*Mastigocladus*) *laminosus* (Table 3.1, Fig. 3.6). Typically, corroborating evidence for their greater breadth of distribution also exists from the microscopic observation of environmental samples and from culture collections (see above).

There are good reasons, however, to believe that dispersal barriers to migration exist even for the most widely distributed thermophilic cyanobacteria. Miller et al. (2007) observed a strong positive correlation between the amount of genetic differentiation between populations of the cosmopolitan bacterium *F. laminosus* and the physical distance which separates them. This result meets the prediction of the isolation-by-distance model (Wright 1943), in which the probability of dispersal decreases with increasing distance. Miller et al. (2006) explicitly estimated the migration rates between relatively close White Creek and Boiling River, YNP populations of *F. laminosus*, which are separated by approximately 50 km. These populations exhibit considerable genetic differentiation based on sequence data from four loci involved in nitrogen metabolism (*nifH*, *narB*, *nirA*, *devH*), with no shared genotypes between them. However, the degree of genetic differentiation (i.e., $_{FST}$) of these populations could in principle be explained by an equilibrium between migration and genetic drift, rather than simply by geographic isolation (i.e., the absence of migration). Using the isolation-with-migration model (Nielsen and Wakeley 2001; Hey and Nielsen 2004) to distinguish between these possibilities, migration between the populations was undetectable, supporting the conclusion that the White Creek and Boiling River populations were recently geographically isolated (Miller et al. 2006).

Although these results suggest that successful migration across even moderate distances should be considered an extremely low probability event, even for "strong" dispersers, these events still clearly happen on a contemporary time scale. For example, identical multi-gene haplotypes of *F. laminosus* have been recovered from geographically distant locations including Montana (USA), Chile and Oman (Miller et al. 2007; Ionescu et al. 2010). A possible explanation for the geographically wide distribution of these *Fischerella* genotypes, is the fact that this cyanobacterium tolerates desiccation and freezing, produces akinete-like cells and also grows slowly even at non-thermal temperatures (25–30°C), which may allow it to exist in many "stepping stone" aquatic habitats.

3.3.2 Distribution Along Effluent Flow Path

Although chemistry may also change as water flows away from the source pool, temperature is a primary organizer of cyanobacterial diversity along hot spring outflow channels. The temperature dependence of the distributions of *Synechococcus* A-like and B-like cells in Octopus Spring (Ferris and Ward 1997; Ward et al. 1998; Ward and Castenholz 2000) and Mushroom Spring (Ward et al. 2006 based on denaturing gradient gel electrophoresis (DGGE) banding patterns remains a prime example of ecotypic differentiation in the microbial world, with members of the A-like and B-like groups generally more abundant at higher and lower temperatures, respectively. Specifically, 16S rRNA genotype variants A″, A′, A, B′ and B are distributed progressively from ~72–74°C to ~50°C along the effluent flow path (see Figure 4 of Ward and Castenholz 2000). Similarly, A-like and B-like 16S rRNA genotypes were retrieved by PCR amplification and cloning from ~60°C to ~50°C regions of Hunter's Hot Springs, OR (Papke et al. 2003).

More recently, DGGE has also been used to investigate the cyanobacterial diversity of the microbial mats that develop at lower mean temperature than that mentioned above. Norris et al. (2002) observed largely similar communities for 40–47°C mats from Octopus Spring and a tributary of Rabbit Creek, respectively, including members of the *Synechococcus* C9 and OS Type P groups (see Ferris et al. 1996). Lau et al. (2005) specifically examined topographical patterns of diversity of and around the raised structures at Black Sand Pool, Yellowstone NP. These structures, believed to be produced primarily by phototactic filamentous cyanobacteria, are often conspicuous features of alkaline hot spring microbial mat communities at lower temperature (Fig. 3.2f). As found by Norris et al. (2002), the raised structures contained *Synechococcus* OS Type P and C9 cells.

An additional DGGE band sequence was related to a filamentous cyanobacterium that has been isolated in laboratory culture from low temperature mats from Octopus Spring (Norris et al. 2002; cyanobacterium OLI-01) and a hot spring in Fairy Creek meadows, NP (Bosak et al. 2009; *Leptolyngbya* sp. FYG). Two other DGGE band sequences appear to belong to the *Synechococcus* "T1" sister group of the *Synechococcus* A/B clade (see above). In contrast, members of the *Synechococcus* B group were recovered from the surrounding nonlithified mat.

Since the publication of Ward and Castenholz (2000), advances in high-throughput DNA sequencing technologies (e.g., Margulies et al. 2005) have enabled more extensive sampling of spatial distribution of microbial diversity along thermal gradients. Miller et al. (2009a) employed a barcoded pyrosequencing approach using PCR amplification to target the V3 region of the bacterial 16S rRNA gene to investigate community structure along the thermal gradients of White Creek and Rabbit Creek. It should be noted that a limitation of using the V3 sequence is reduced phylogenetic resolution for some taxa, particularly among members of the diverse *Synechococcus* B group, which have identical sequences in this region. Approximately one-third of the nearly 34,000 total sequences collected from ten sites along each gradient were of cyanobacterial origin. Of these, a combined total of 41 unique V3 sequence signatures (termed operational taxonomic units (OTUs) in Miller et al. 2009a) were recovered from the two streams. By contrast with what was found for Mushroom Spring above, the FAPs were almost exclusively *Chloroflexus* spp. along the entire White Creek thermal gradient (Miller et al. 2009b).

As expected, A-like *Synechococcus* lineages dominated at the highest temperature sites, with the same two OTUs together comprising a majority of the entire microbial communities at both creeks (Fig. 3.7). In White Creek, B-like *Synechococcus* lineages dominated between 58°C and 64°C. In Rabbit Creek, the 63–64°C region of the thermal gradient was primarily occupied by a member of the A-like *Synechococcus* group (OTU 53) that was very rare at White Creek. The breadth of the realized distribution of the B-like *Synechococcus* OTU also differed between channels. At Rabbit Creek, these were the most abundant cyanobacteria between approximately 47°C and 61°C, whereas, at White Creek, *Fischerella laminosus* and OS Type P-like *Synechococcus* predominated at temperatures below about 55°C (Fig. 3.7) (Miller et al. 2009a). The springs differ greatly in the availability of combined nitrogen at temperatures below ~55°C (Miller et al. 2009b), and this may help to explain the presence of the heterocyst-forming, nitrogen-fixing *F. laminosus* at White Creek (instead of B-like *Synechococcus* populations) and its apparent absence at Rabbit Creek.

The clearly resolved range boundaries among different *Synechococcus* lineages provide additional insights into the ecological interactions among these bacteria. The realized niches observed for *Synechococcus* OTUs are more narrow than the temperature ranges permissive for growth of related strains in the laboratory (Miller and Castenholz 2000; Allewalt et al. 2006), and this disparity between the realized and potential niches of representative strains can be attributed to competition in regions of overlap.

At Rabbit Creek, the lowest temperature site was dominated by an unidentified filamentous cyanobacterium that was also detected in Octopus Spring lower temperature mat (Norris et al. 2002; DGGE bands OL6 and OL7). While other taxa were comparatively rare, significant abundances (<1–5% of the total microbial community) for OS Type I, OS Type P and *Synechococcus* C9 groups were observed at some sites (Fig. 3.7).

3.4 Ecology of Well-Studied Hot Spring Cyanobacterial Communities

3.4.1 Mushroom Spring and Octopus Spring *Synechococcus* Mats

The 50°C to 73–74°C *Synechococcus* mats of alkaline siliceous hot springs have been studied for many decades and serve as model systems for understanding the composition, structure and function of microbial communities (Brock 1978; Ward et al. 1998, 2006, 2008, 2011, 2012; Ward and Castenholz 2000). Sections 3.2.3.2 and 3.3.2 showed the positioning of these communities relative to other cyanobacteria found at lower temperatures in these and other springs and introduced the *Synechococcus* A/B-lineage 16S rRNA genotypes that predominate. Here, we update our understanding of linkages among genetic, taxonomic and functional diversity based on studies since Ward and Castenholz (2000).

3.4.1.1 Evidence of Adaptive Differences Among Isolates Relevant to These Mat Systems

The distribution of closely related 16S rRNA variants along the effluent flow path (genotypes A″, A′, A, B′ and B from ~72–74°C to ~50°C (Fig. 3.8a); Ferris and Ward 1997; Ward et al. 2006; Miller et al. 2009a) and vertical dimension (at ~60°C, genotype B′ above genotype A; Ramsing et al. 2000; see Figure 6 in Ward and Castenholz (2000)) led to the hypothesis that *Synechococcus* spp. with these genotypes likely arose through adaptation to parameters that vary along the associated gradients of temperature, light and chemistry (Ward 1998). Allewalt et al. (2006) succeeded in cultivating *Synechococcus* strains with A, B′ and B 16S rRNA genotypes (Table 3.1) and used them to test the hypothesis of adaptation by demonstrating that their temperature preferences were consistent with the *in situ* distributions of

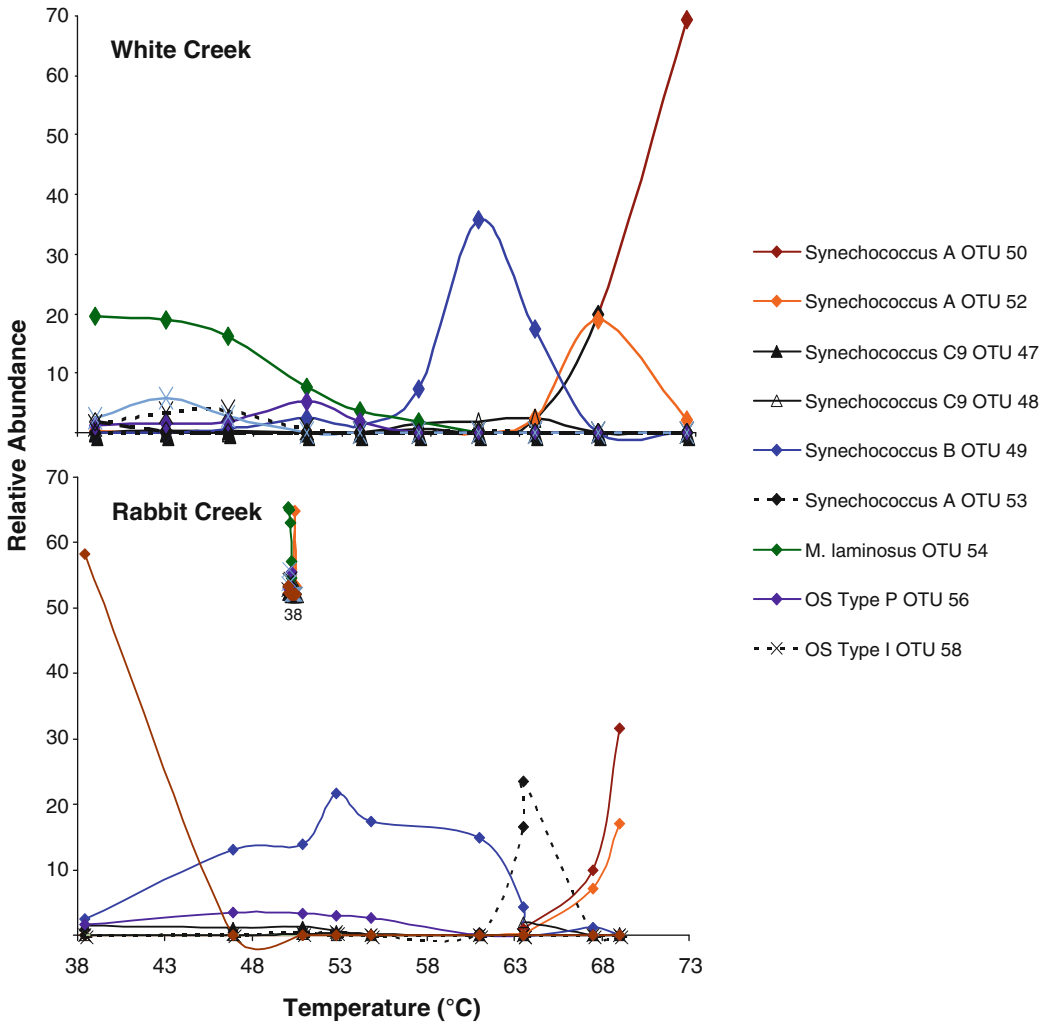


Fig. 3.7 Distribution of major cyanobacterial taxa along the thermal gradients of White Creek and Rabbit Creek. Relative abundance refers to the percentage of sequences of an OTU recovered from a particular environmental sample (Data from Miller et al. 2009b)

their 16S rRNA genotypes (Fig. 3.4c). As mentioned above, Miller and Castenholz (2000) had already demonstrated this for Oregon A/B-like *Synechococcus* strains. Molecular evidence of adaptation has been observed among the Oregon strains for the Calvin cycle enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). The RuBisCO enzyme of *Synechococcus* strain OH28, which is capable of growth at 70°C (Miller and Castenholz 2000), exhibits enhanced thermostability (~7°C greater estimated denaturation temperature in circular dichroism thermal scans) relative to either the native RuBisCOs of less thermotolerant strains or inferred ancestral *Synechococcus* RuBisCOs constructed by sequential site-directed mutagenesis (Miller, unpublished data).

Complete genome sequences have been obtained for *Synechococcus* spp. strains A and B′ (Bhaya et al. 2007) and comparative analyses revealed differences in nutrient acquisition and storage. Specifically, the B′ strain has genes that enable it to use phosphonate as a source of phosphorus (Adams et al. 2008), as well as genes for producing and degrading cyanophycin, which may be involved in storage of nitrogen. These observations may foretell of decreasing availability of phosphate and fixed nitrogen forms downstream in the effluent channel. Genomic analyses also revealed that both strains have the potential to fix N_2, which is interesting in light of the prior work of Stewart (1970) and Wickstrom (1980), which led these authors to the suggestion that *Synechococcus* in such mats do not have the capability to fix N_2. *In situ* expression of *nif* genes and proteins and nitrogen fixation was demonstrated (Steunou et al. 2006, 2008) and will be described in more detail below.

Synechococcus populations at the mat surface (Fig. 3.5a) have distinctly different autofluorescence than those residing ~400–700 μm beneath the mat surface suggesting acclimation

and/or adaptation to lower light intensity and/or differences in light quality (Fig. 3.5b, c). The observations by Ramsing et al. (2000) of (i) the co-occurrence of a genetically distinct *Synechococcus* population (16S rRNA genotype A) with the deeper, more fluorescent population at a ~60° mat site, and (ii) unique cell reorientation of the deeper population at the brightest part of the light cycle, provided further evidence consistent with the hypothesis of differential light adaptation. The light relations of B-, B′- and A-like *Synechococcus* isolates have been studied. While all isolates could photosynthesize at up to 1,100 µmol photon m^{-2} s^{-1} and grow at up to at least 385 µmol photon m^{-2} s^{-1} (Allewalt et al. 2006), the B′ isolate was reportedly unable to maintain growth for more than 5 days at 400 µmol photon m^{-2} s^{-1} (Kilian et al. 2007). Even at 200 µmol photon m^{-2} s^{-1} this isolate reduced levels of phycobilisomes and chlorophyll and increased levels of carotenoids. The interpretation of these results is complex. The *Synechococcus* sp. strain B′ isolate might live in the upper photic zone and tolerate high light intensity. *In situ* it never experiences continuous high light for many days. Furthermore, surface *Synechococcus* populations appear to shut down oxygenic photosynthesis in the brightest part of the day (Ramsing et al. 2000). Alternatively, this strain might reside in lower parts of the photic zone and be low-light adapted. B′-like *Synechococcus* populations are also found deeper in the 60°C mat (Ramsing et al. 2000; Becraft et al. 2011) and there is evidence that photosynthesis maximizes in deeper layers at the highest light intensities (Ramsing et al. 2000). Clearly, more information about growth as a function of light intensity and/or quality is needed to resolve whether light adaptations occur in thermophilic *Synechococcus* spp.

3.4.1.2 Nature of *Synechococcus* Species in These Mats

At 65°C and 68°C sites, single 16S rRNA genotypes A and A′, respectively, occur at all depths in the photic zone (Ward et al. 2006). The question arose as to whether the phenotypically distinct *Synechococcus* populations at different mat depths at these temperatures (Fig. 3.5a) represented a single genetic population (i.e., species) acclimated to differing environmental conditions or >1 species adapted to different vertical microenvironments and so closely related that they could not be resolved using the slowly evolving 16S rRNA molecule. Ferris et al. (2003) showed that a more rapidly evolving genetic locus, the internal transcribed spacer (ITS) sequence separating 16S and 23S rRNA genes, could resolve the A′-like *Synechococcus* populations occurring at 68°C into two, more closely related genetically distinct populations, which occur at surface or sub-surface depths. However, at ~65°C the differently fluorescing A-like *Synechococcus* populations could not be resolved by either 16S rRNA or ITS sequence variation. These observations raised the question of how much molecular resolution is needed in order to discern all *Synechococcus* species in the mats. We have examined many protein-encoding genes in both single-locus and multi-locus analyses to address this question, as will be described below. However, to understand the results of these analyses, it is necessary to first consider the central question of how individual genetic variants are grouped into species populations. The realization that the origin of differently adapted *Synechococcus* species in these mats is best described by the Stable Ecotype Model of species and speciation (Ward and Cohan 2005) precipitated the development of approaches that have provided a way to answer to this question.

In the Stable Ecotype Model (Cohan and Perry 2007), ecotypes, known also as ecological species (*sensu* Van Valen 1976), are conceptualized as populations of ecologically interchangeable variants that differ genetically because of ecologically neutral mutation and/or recombination events that accumulate uniquely in organisms occupying distinct niches. Ecotypes evolve as natural selection periodically favours the most-fit variant among those capable of inhabiting a niche. Periodic selection thus reduces genetic diversity within an ecotype, which rises anew as ecologically neutral mutations accumulate in the survivor of the most recent periodic selection event. When variants arise that are ecologically different than other variants in the ecotype, they initiate new populations because they are not affected by the periodic selection events affecting the parent population; they diverge and eventually evolve into a new and distinct ecological species (i.e. ecotypes). Such populations can be demarcated from molecular sequence variation, since each species accumulates different genetic variants. Koeppel et al. (2008) developed a Monte Carlo evolutionary simulation (Ecotype Simulation) based on the Stable Ecotype Model that determines the order and frequency of periodic selection and ecotype formation events and the number of ecotypes that best explains the evolution of a lineage as reflected by gene phylogenies. Consequently, this algorithm predicts how individual molecular variants are grouped into ecological species populations. A test of Ecotype Simulation using the ITS results mentioned above demonstrated that the putative *Synechococcus* ecotypes predicted by Ecotype Simulation do have distinctive ecology (i.e. unique distributions along flow and vertical gradients; (Fig. 3.8b) see Ward et al. 2006). Ecotype simulation analysis of protein-encoding loci has revealed the existence of on the order of 13–14 ecological species of *Synechococcus* within both the A and B′ 16S rRNA genotypes, and fine-scale distribution patterns, gene expression patterns and population dynamics in response to environmental alteration confirm the ecological distinctions of these predicted ecotype populations (Melendrez et al. 2011; Becraft et al. 2011; Ward et al. 2011) (Fig. 3.8c). The number of predicted ecotypes rises with extent of sampling of a population and with the molecular resolution of

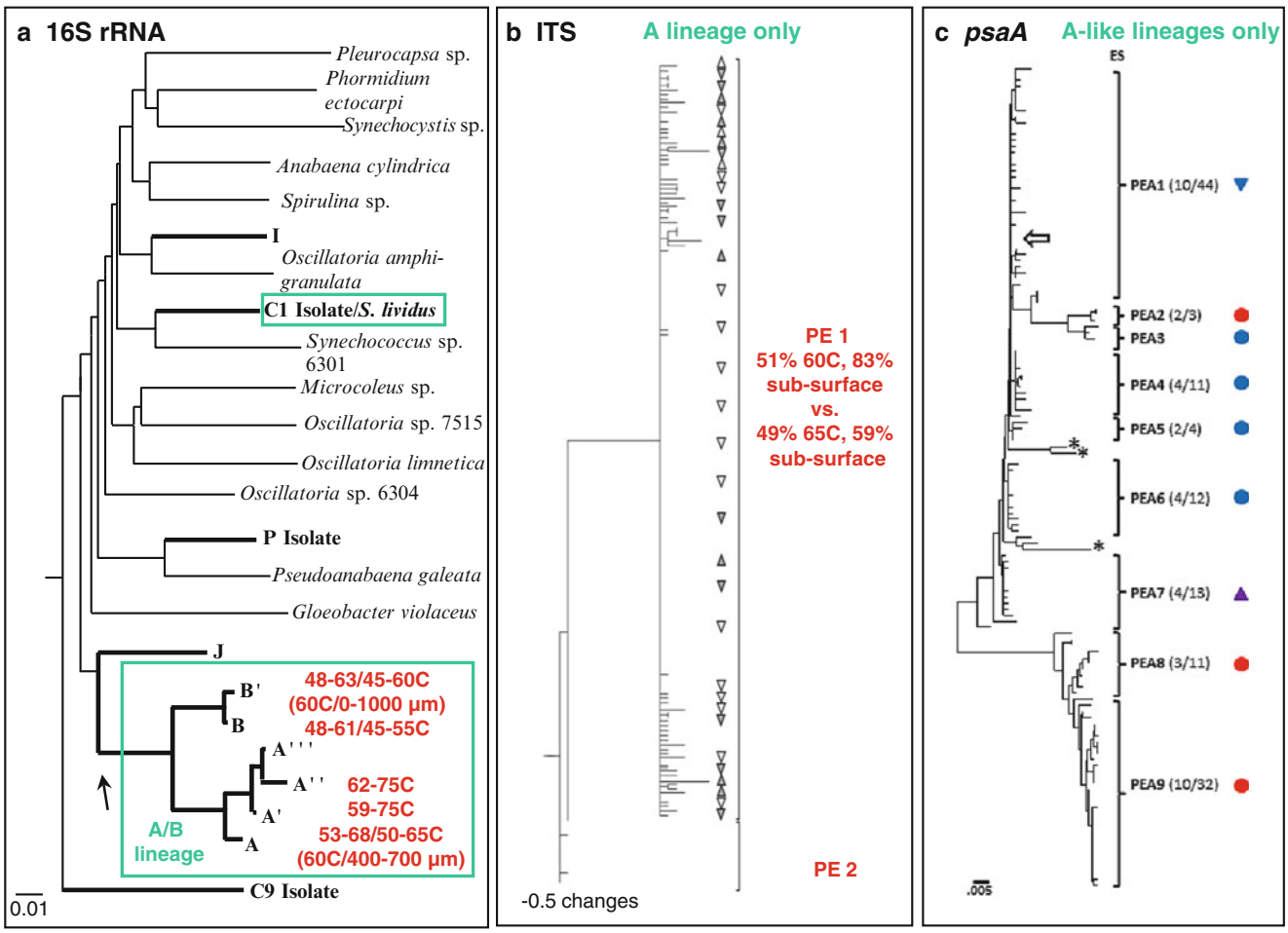


Fig. 3.8 Phylogenetic trees showing *Synechococcus* putative ecotypes (*PE*) detected by Ecotype Simulation analysis of sequence data: (**a**) Partial 16S rRNA gene; (**b**) 16S–23S rRNA ITS sequence; (**c**) Partial *psaA* gene. *Red text* indicates information about temperature and depth distribution of PEs. *Green boxes* in panel **a** highlight the *Synechococcus* A/B lineage and the sequence of the readily cultivated *S. lividus*. In (c) colors indicate temperatures (blue, 60°C; purple, 63°C; red, 65°C) and triangles point toward surface and sub-surface populations. (Data from Ward et al. 1998, 2006; Becraft et al. 2011)

the gene studied (Melendrez et al. 2011). Ecotypes are also detected in cultivation-independent multilocus sequence analysis, despite evidence that recombination exceeded mutation during the evolution of the *Synechococcus* A/B lineage (Melendrez et al. 2012). We estimate that between ~60°C and 65°C sites there are on the order of 30 *Synechococcus* ecotypes (i.e., ecological species), which are concealed by common morphology and 16S rRNA genotypes.

3.4.1.3 Metagenomic Analysis of Community Composition

Metagenomic sequences were obtained from the upper cyanobacterial layers of ~60°C, ~65°C and ~68°C regions of the Mushroom Spring mat and ~60°C and ~65°C regions of the Octopus Spring mat (Bhaya et al. 2007; Klatt et al. 2011). Based on phylogenetic similarity and oligonucleotide frequency analyses, these sequences assembled into eight well-defined clusters (Fig. 3.9a), one of which corresponds to A-like and B′-like *Synechococcus* populations. This population comprises about 30% of the genes in the metagenomes and, as in 16S rRNA analyses, A- and B′-like populations predominate at 65°C and 60°C, respectively (Klatt et al. 2011). Comparative analysis of these metagenomes and the *Synechococcus* spp. strain A and B′ genomes revealed that the isolates were closely related to native populations (Fig. 3.9b). Metagenomic clones with only one end sequence that was closely associated with the *Synechococcus* spp. strain A or B′ reference genome revealed (through the sequences at the other end of the same clone) differences

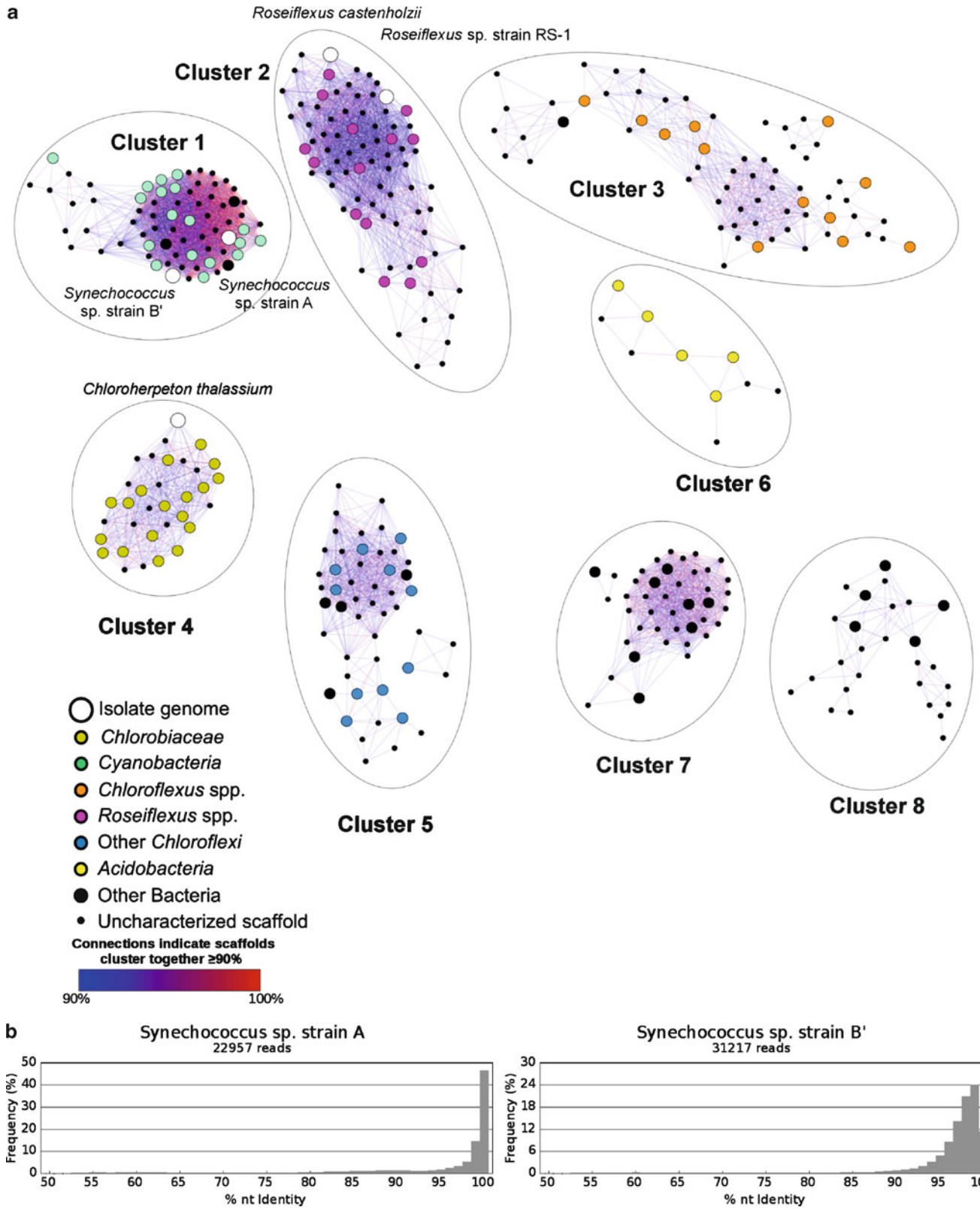


Fig. 3.9 Metagenomic analysis of 60°C and 65°C regions of Mushroom Spring and Octopus Spring mat top green layers: (**a**) Network map of core scaffold clusters observed in Celera assemblies. Scaffolds with similar oligonucleotide frequency profiles that group together in the same cluster in ≥90% of 100 trials are indicated by *connecting lines*, whose color reports the percentage that scaffolds group together as defined by the *scale bar*. Isolate genomes included in this analysis are indicated by *large white circles*, whereas metagenomic scaffolds that contain characterized phylogenetic marker genes are marked as *medium-sized circles* colored according to taxonomic grouping. *Large ovals* were drawn by hand to demarcate the different clusters. (**b**) Histograms of metagenomic sequences that can be associated confidently with the *Synechococcus* sp. strain A (*left*) or strain B′ (*right*) reference genome presented as a function of their % NT ID relative to the reference genome that recruited them in BLASTN analysis (From Klatt et al. 2011)

between cultivated and native *Synechococcus* populations. For instance, both A-like and B′-like populations appear to include organisms, which, unlike the cultivated strains, possess *feo* genes that might confer the ability to incorporate ferrous iron (Bhaya et al. 2007; Klatt et al. 2011). Fe^{2+} acquisition could be a heretofore unsuspected niche-determining characteristic. Genes closely related to those of other thermophilic cyanobacteria (e.g. *Thermosynechococcus elongates*, a member of the C1 *Synechococcus* lineage: see Papke et al. 2003) were not detected. This was consistent with their low abundance in Yellowstone cyanobacterial mats (Papke et al. 2003; Miller et al. 2009a; see Fig. 3.7).

Five of the other metagenomic clusters correspond to anoxygenic phototrophic bacteria, including *Roseiflexus*, *Chloroflexus*, a member of the Chlorobiales, *Candidatus* Chloracidobacterium thermophilum and a novel phototroph that is phylogenetically deep within Kingdom Chloroflexi (Klatt et al. 2011). Two other clusters appear to represent heretofore unknown community members that, based on current analyses, do not appear to contain genes associated with phototrophic metabolisms. Clearly, the complexity of phototrophs and other dominant members of these mat communities is greater than previously thought, especially when it is clear that these clusters may, like the *Synechococcus* cluster, contain numerous closely related species.

Metagenomes have also been obtained for the Mushroom Spring 60°C undermat and other Yellowstone geothermal features dominated by cyanobacteria, including, White Creek and Chocolate Pots Spring (Klatt et al. 2012b). These databases are beginning to broaden our understanding of connections between potential function and cyanobacteria in these geochemically different habitats.

3.4.1.4 *In Situ Synechococcus* Physiology

Oxygen microsensors have been used extensively to document oxygenic photosynthesis and the resulting oxygen profiles relative to the *in situ* realities of light in these mats (e.g. Ward et al. 2006) (Fig. 3.5b, c). Mats at temperatures ≤65°C have such compressed photic zones (Fig. 3.5a) that vertical profiles of oxygenic photosynthesis usually show only one depth at which photosynthesis maximizes (Fig. 3.5c). However, the more translucent mats at ~68°C show evidence of possible multiple photosynthetic maxima at different depths (Fig. 3.5c). Distinct photosynthesis maxima at different depths was also observed during a period of recovery following removal of the upper green mat layer, when light was more available for deeper populations (Ferris et al. 1997). These observations suggest that distinct *Synechococcus* populations conduct photosynthesis at different depths, consistent with ecotype distribution results. As mentioned above, Ramsing et al. (2000) suggested that the vertical positioning of oxygenic photosynthesis (estimated from vertical oxygen profiles) moves to deeper mat layers as light intensity increases during the morning and this suggests that species residing at different depths in the photic zone are engaged in photosynthesis at different times of day. Spectral alteration of light that penetrates into the mat (Fig. 3.5b) may suggest adaptation or acclimation to light quantity and/or quality.

It was previously demonstrated using microsensors that (i) the *Synechococucs* spp. photosynthesize so intensely that their CO_2 consumption causes pH to rise from 8.4 in water flowing above the mat to ~9.4 (Revsbech and Ward 1984) and (ii) using $^{14}CO_2$ labeling, that these low CO_2/high O_2 microenvironmental conditions lead to photorespiratory production of glycolic acid, which may be transferred to other mat organisms (Bateson and Ward 1988). Other $^{14}CO_2$ labeling studies showed that mat *Synechococcus* populations produce mainly polyglucose as a consequence of oxygenic photoautotrophy during the day and shift their metabolism to polyglucose fermentation in the dark (Konopka 1992; Nold and Ward 1996), mainly to acetate, propionate and CO_2. Based on evidence of the photoassimilation of fermentation products by filamentous mat inhabitants (Sandbeck and Ward 1981; Anderson et al. 1987), it was hypothesized that *Synechococcus* spp. cross-feed fermentation products to *Roseiflexus* spp. and/or *Chloroflexus* spp. (Nold and Ward 1996) (Fig. 3.10a). This was demonstrated by stable isotope probing of diagnostic lipid biomarkers (van der Meer et al. 2005). In these experiments the mat was pulse-labeled with $^{13}CO_2$ during the afternoon, resulting in labeling of *Synechococcus* spp. lipids (and presumably polyglucose based on $^{14}CO_2$ labeling results mentioned above). After incubation during the night and into the following morning, lipids diagnostic of *Roseiflexus* spp. became labeled and the labeling could be blocked by increasing the pool size of unlabeled acetate and glycolate (Fig. 3.10b). Previous natural abundance stable isotope studies had demonstrated that *Roseiflexus* spp. lipid biomarkers were isotopically heavier than expected based on photoheterotrophic uptake of fermentation products derived from cyanobacteria, which use the Calvin-Bensen Cycle for fixing CO_2 (van der Meer et al. 2000, 2003). *Roseiflexus* sp. strain RS1, which is closely related to native *Roseiflexus* populations (van der Meer et al. 2010), was found to possess genes for photoautotrophy using the 3-hydroxypropionate cycle (Klatt et al. 2007). This pathway has a lower degree of isotopic fractionation than the Calvin-Bensen Cycle and could explain the heavier *Roseiflexus* spp. lipid biomarker signatures. van der Meer et al. (2007) showed the natural cycling of polyglucose in mat *Synechococcus* spp., which had been partially separated from other mat inhabitants using a Percol gradient (Fig. 3.11a). This separation enabled demonstration that the stable carbon isotopic fractionation in polyglucose biosynthesis is much lower than in lipid biosynthesis. Hence, the transfer of carbon, first fixed as polyglucose and then fermented by *Synechococcus* spp., coupled to the uptake of fermentation products by *Roseiflexus* spp. may also explain the heavy isotopic signatures in *Roseiflexus* spp. lipids. Recent evidence from diel metatranscription patterns, suggest that *Roseiflexus* spp. may use the 3-hydroxypropionate pathway in a mixotrophic, rather than an autotrophic process (Bryant et al. 2011; Klatt et al. 2012a).

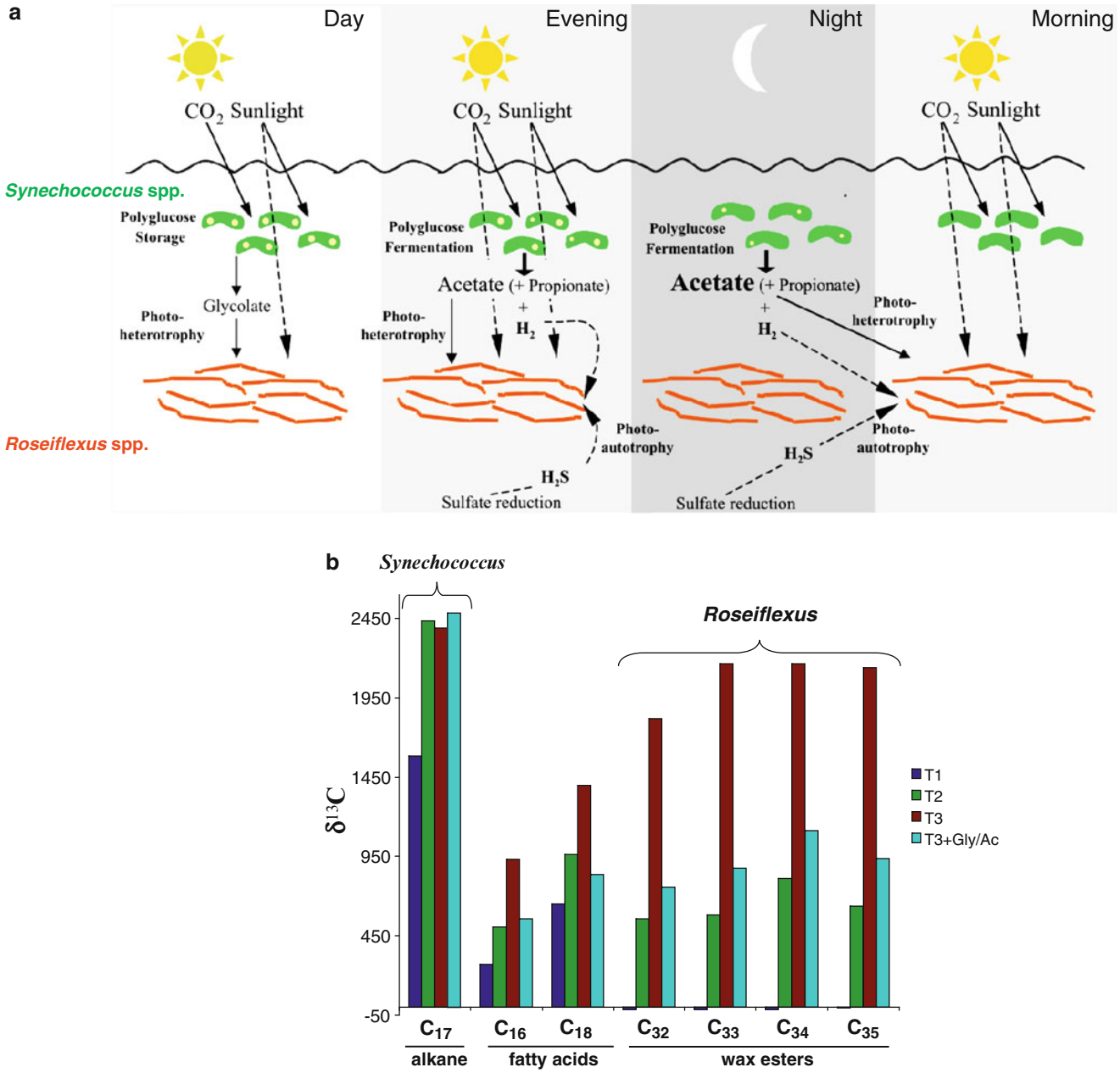
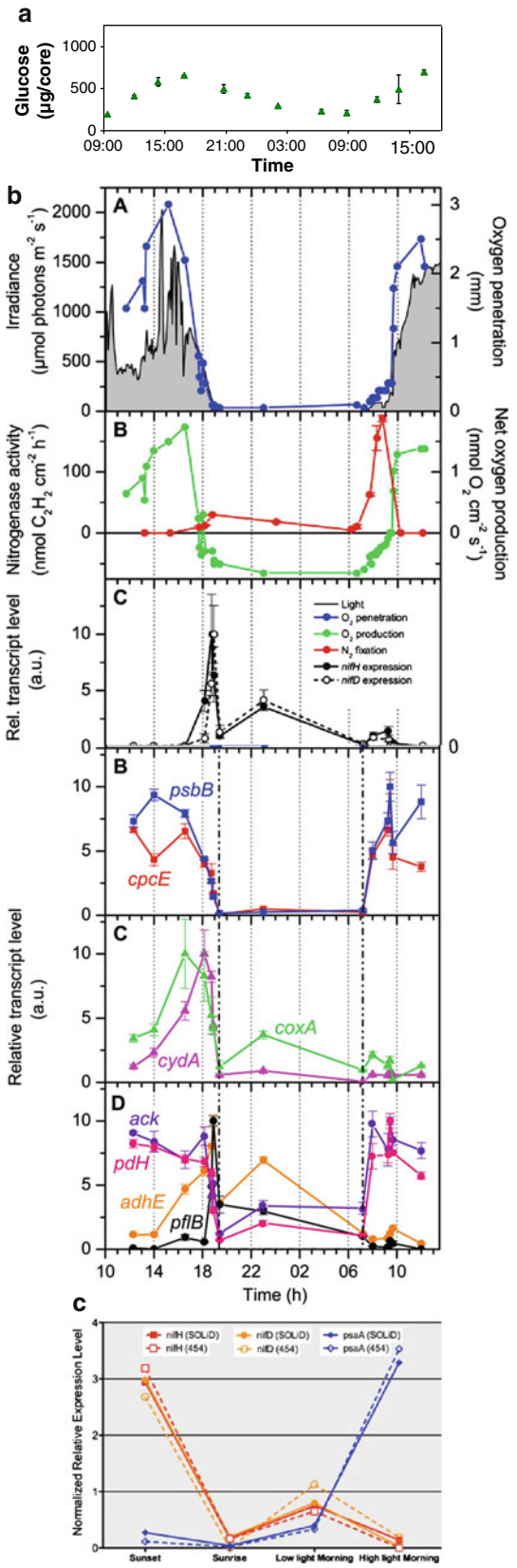


Fig. 3.10 **Cross feeding of metabolites from *Synechococcus* spp. to *Roseiflexus* spp.:** (**a**) Model postulating midday *Synechococcus* production of glycolic acid and polyglucose followed by a shift to fermentation at night and uptake of fermentation products in the morning by *Roseiflexus* spp. (**b**) Incorporation of $^{13}CO_2$ into diagnostic *Synechococcus* and *Roseiflexus* lipids in the afternoon (*T1*, 1,720 h), then, after removal of unmetabolized $^{13}CO_2$, redistribution of fixed ^{13}C resulting from continued incubation under natural light conditions until early morning (*T2*, 0645 h) and late morning (*T3*, 1055 h). Labeling between *T2* and *T3* was done with and without addition of a mixture of unlabeled glycolate and acetate at *T2* (*T3* + Gly/Ac) (Modified from van der Meer et al. 2005)

3.4.1.5 *In Situ* Gene Expression and Diel Metabolic Shifts

Steunou et al. (2006, 2008) used quantitative reverse transcriptase PCR to study the *in situ* transcription of B′-like *Synechococcus* genes in the ~60°C mat. These studies were focused on the transcription of genes associated with (i) nitrogen fixation, the genetic potential for which was discovered in the genomic analyses described above, and (ii) energy metabolisms that might drive this process throughout the diel light cycle. Interestingly, *nif* genes are transcribed in the evening (Fig. 3.11bC) when oxygenic photosynthesis no longer exceeds aerobic respiration and the mat becomes anoxic, except in the surface ~100 μm (Fig. 3.11bA). The upregulation of genes associated with aerobic respiration may indicate that *Synechococcus* spp. actively scavenge oxygen in the late afternoon (Fig. 3.11bE). Nif proteins are also produced and nitrogenase activity initiates (Fig. 3.11bB) in the evening. However, rates of N_2 fixation are low until morning, when a



burst of activity is observed. The low nighttime rates of N_2 fixation are presumably due to the low energy yield provided by polyglucose fermentation. The morning burst of N_2 fixation occurs when the mat receives diffuse light and remains anoxic because aerobic respiration exceeds photosynthetic oxygen production (Fig. 3.11bB). Once direct sunlight illuminates the mat, oxygen accumulates and *nif* gene transcripts and proteins and N_2 fixation, can no longer be detected. Genes involved in photosynthesis (Fig. 3.11bD) and some fermentation genes (Fig. 3.11bF) are expressed in a pattern consistent with the shift between these metabolisms in light and dark periods. Thus, it appears that *Synechococcus* spp. play a major role in the nitrogen economy and in recycling photosynthetically fixed carbon in the mat community. Jensen et al. (2010) demonstrated the *in situ* diel dynamics of the expression of B′-like *Synechococcus* genes associated with intense consumption of inorganic carbon (and resultant pH increase) and production of oxygen during oxygenic photosynthesis by such dense populations as occur in these mats. Transcripts of genes involved in both carbon concentration and reactive oxygen protection increased during the morning light transition and declined after the mid-day light, O_2 and pH maxima.

Metatranscriptomic analyses conducted using pyrosequencing and SOLiDTM sequencing confirm these targeted gene analyses (Fig. 3.11c), showing patterns of expression of N_2 fixation and photosynthesis genes that were nearly identical to those observed in the targeted analyses reported above (Liu et al. 2011). These global analyses will permit, in the very near future, detailed investigation of *in situ* physiology of *Synechococcus* spp. and other mat inhabitants. Resolution of A-like and B′-like *Synechococcus* transcription appears possible with such analyses (Liu et al. 2011). Targeted reverse-transcriptase PCR analysis of the *psaA* gene appears to permit analysis of species-specific transcription and these patterns are also seen in initial Ti454 metatranscriptomes (Becraft et al. 2012). Metatranscriptomic and metaproteomic analyses of samples collected at hourly intervals through a complete diel cycle are in progress (Liu et al. 2012 Ward, D.A. Bryant, L.Steinke,

Fig. 3.11 Diel metabolic rhythms in Mushroom Spring 60°C mat *Synechococcus* spp.: (**a**) Polyglucose levels associated with mat *Synechococcus* cells partially purified by Percol gradient separation (From van der Meer et al. 2007). (**b**) Light intensity and oxygen penetration (*A*), net oxygen production and nitrogenase activity (*B*), B′-like *Synechococcus* sp. gene transcripts involved in N_2 fixation (*C*), photosynthesis (*D*), aerobic respiration (*E*) and fermentation (*F*) (From Steunou et al. 2008) (**d**) Metatranscriptomic results for expression of N_2 fixaton (*nifH*, *nifD*) and photosynthesis (*psaA*) genes in samples collected at sunset, sunrise and at low-light and high-light morning periods (Data from Liu et al. 2011)

M.Lipton, unpublished data) and these should yield detailed information about the timing of gene expression and protein abundance. For any transcripts that offer as much molecular resolution as the *psaA* gene (and possibly some proteins), it may be possible to understand the functional ecology of *Synechococcus* spp. and other mat inhabitants at the species level.

3.4.2 White Creek *Fischerella*

F. laminosus is the predominant cyanobacterium at White Creek between approximately 39°C and 54°C mean annual temperature (Fig. 3.7). Recent efforts have provided insights into the distribution of genetic and ecological variation in this population (Miller et al. 2006, 2009b; Miller 2007, 2010) that complement work on thermal niche differentiation of the *Synechococcus* A/B group.

Although population members distributed along the White Creek thermal gradient exhibit no divergence at the 16S rRNA locus or the adjacent ITS region (Miller et al. 2006), they do harbor low amounts of genetic variation (~1 polymorphic site per kb) in other, less conserved, regions of the genome (Miller et al. 2006, 2009b). Extensive recombination observed among these variable markers (Miller et al. 2006, 2007, 2009b) acts to break up the genetic linkage that would otherwise result in genome-wide nonrandom associations between alleles at different loci. Most of this variation exhibits little or no distribution difference along the channel (Miller et al. 2009b; Miller 2010, unpublished data), which suggests a highly genetically-connected population that is not limited by dispersal. However, some variable markers (e.g. *nifH*, *rfbC*) show strong genetic differentiation (i.e. high F_{ST}) along the White Creek thermal gradient, with different alleles predominating at upstream and downstream zones, respectively (Miller et al. 2009b; unpublished data). A possible explanation for the above patterns of genetic polymorphism is that migratory gene flow is generally unrestricted in the population but is prevented in certain regions of the genome by natural selection.

Temperature is likely to be a selective agent that contributes to these barriers to gene flow, since genetically divergent strains of *F. laminosus* exhibit differences in thermal performance that closely match the prevailing conditions that they experience *in situ* (Miller et al. 2009b). Population members can therefore be considered to be ecological specialists that have diverged in their thermal niches. Crossing reaction norms between high temperature (55°C) and low temperature (37°C) performance among strains indicates that a trade-off at physiological extremes has likely contributed to niche differentiation of the population. Ongoing work seeks to identify the functionally important genetic variation that underlies these ecological differences.

3.5 Conclusions

Ward and Castenholz (2000) expressed the hope that the results produced by mentors studying cyanobacteria in geothermal habitats would, like those of the masons who envisioned the great cathedrals of Europe, but could not witness their completion, provide a solid framework for further subsequent independent research by their students and others. Happily, this has occurred, though not in exact terms.

An example of consistency can be found in patterns of temperature adaptation, which have been observed as new cyanobacterial isolates have been brought into culture, which are known to be genetically representative of the native populations from which they were obtained. Their temperature relations in culture (fundamental niche) have been shown to correlate with their *in situ* distributions (realized niche), and we are beginning to develop an understanding of how the evolution of physical properties of macromolecules have shaped observed differences in temperature relations. However, an example of inconsistency can be found in differences in the importance of particular types of cyanobacteria at particular temperatures in particular hot springs as revealed in high-throughput molecular analyses. For instance, *Synechococcus* genotypes have been seen to exhibit different temperature-defined realized niches in different hot spring effluents. Differences have also been noted in the predominant filamentous anoxygenic phototrophic populations in different hot spring effluent channels. These differences remind us that temperature is not the only determinant of niche. Indeed, genomic and metagenomic analyses have revealed nutritional differences suspected from earlier morphoytype distribution studies. These differences also remind us that the competitiveness of a population depends on all the various niche determinants. Thus, patterns observed in one system may not apply to another system.

A second example of consistency is that higher molecular resolution analyses have confirmed the inference made based on patterns seen in 16S rRNA distribution analyses that adaptation has been important in the evolution of geothermal cyanobacteria. In fact, when resolution is sufficient to view variation among individuals it is possible to conduct population genetics analyses that result in the demarcation of populations of ecologically interchangeable individuals equivalent to ecological species that have unique ecological adaptations. However, insufficient resolution may effectively lump these species populations into sets of populations that appear to be heterogeneous rather than homogeneous with respect to ecological uniqueness. Demarcation of taxa by morphological similarity is dangerous, as the number of species within a morphologically defined taxon can be very large and this can, in turn, obfuscate the

recognition of patterns suggesting adaptive and biogeographical differences. For instance, *Synechococcus* is genetically extremely diverse and exhibits different patterns of global distribution suggestive of divergence due to geographic isolation, some of which would have been masked by defining this genus on the basis of morphological similarity. *Fischerella*, while more genetically coherent, nevertheless also exhibits population structure that indicates that adaptation to temperature and physical isolation are associated with genetic divergence. These observations confirm the generalizations of Castenholz (1992) that "Obviously, cyanobacterial species are the results of natural selection … Also, some species have very restricted geographic distributions, suggesting that allopatric speciation can be important in cyanobacterial evolution." But, his observation that "most species or strains of cyanobacteria seem to have been selected for a wide tolerance of environmental extremes or simply for a great 'ecological amplitude'" may need to be reinvestigated at higher resolution, if based on morphotype distribution patterns.

Well-studied hot spring microbial mat systems continue to serve as excellent models from which to make discoveries of general importance to microbial community ecology. Their extremeness provides relative simplicity, which makes them highly tractable for study by metagenomic, metatranscriptomic and metaproteomic techniques. These methods offer the advantage of being global with respect to their ability to address comprehensively the question of "who is there" in a microbial community. Metagenomic assembly analyses confirm the observations made from more targeted genetic analyses of the predominant cyanobacterial taxa, but have also enabled the discovery of many novel and previously unsuspected noncyanobacterial community members, with whom the cyanobacteria interact. In essence, these "omics" methods have enabled a more objective analysis of a community system, freeing the microbial ecologist from unforeseen inadequacies of traditional approaches and ways of thinking about microbial communities. Metagenomics also provides a means of linking the phylogenetically defined populations to their potential function. Similarly, metatranscriptomics and metaproteomics are beginning to provide global analyses aiming in the direction of community function ("who is transcribing and translating what genes, when and where?"), possibly even to the species level of resolution. When combined with the kinds of labeling experiments that are possible in these relatively simple systems, it may even be possible to associate actual functions with the species responsible. In the future, we expect these systems to be excellent models for understanding the networking among species in the various functional guilds of the community, hopefully providing insights that will be important for microbial systems in more moderate environments that are not so tractable for "omics" analyses.

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References

Adams MM, Gómez-García MR, Grossman AR, Bhaya D (2008) Phosphorus deprivation responses and phosphonate utilization in a thermophilic *Synechococcus* sp. from microbial mats. J Bacteriol 190:8171–8184

Allewalt JP, Bateson MM, Revsbech NP, Slack K, Ward DM (2006) Effect of temperature and light on growth and photosynthesis of *Synechococcus* isolates typical of those predominating in the Octopus Spring microbial mat community. Appl Environ Microbiol 72:544–550

Anderson KL, Tayne TA, Ward DM (1987) Formation and fate of fermentation products in hot spring cyanobacterial mats. Appl Environ Microbiol 53:2343–2352

Anitori RP, Trott C, Saul DJ, Bergquist PL, Walter MR (2002) A culture-independent survey of the bacterial community in a radon hot spring. Astrobiology 2:255–270

Awramik SM, Vanyo JP (1986) Heliotropism in modern stromatolites. Science 231:1279–1281

Banerjee M, Everroad RC, Castenholz RW (2009) An unusual cyanobacterium from saline thermal waters with relatives from unexpected habitats. Extremophiles 13:707–716

Bateson MM, Ward DM (1988) Photoexcretion and consumption of glycolate in a hot spring cyanobacterial mat. Appl Environ Microbiol 54:1738–1743

Becraft ED, Cohan FM, Kühl M, Jensen S, Ward DM (2011) Fine-scale distribution patterns of *Synechococcus* ecological diversity in microbial mats of Mushroom Spring, Yellowstone National Park. Appl Environm Microbiol 77:7689–7697

Becraft ED, Cohan FM, Wood JM, Ward DM (2012) Pyrosequencing analyses of *Synechococcus* ecological species inhabiting the microbial mat of Mushroom Spring, Yellowstone National Park (in preparation)

Bhaya D, Grossman AR, Steunou A-S, Khuri N, Cohan FM, Hamamura N, Melendrez MC, Nelson W, Bateson MM, Ward DM, Heidelberg JF (2007) Population level functional diversity in a microbial community revealed by comparative genomic and metagenomic analyses. ISME J 1:703–713

Bosak T, Liang B, Sim MS, Petroff AP (2009) Morphological record of oxygenic photosynthesis in conical stromatolites. Proc Natl Acad Sci USA 106:10939–10943

Brock TD (1973) Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. Science 179:480–483

Bryant DA, Klatt CG, Frigaard N-U, Liu Z, Li T, Zhao F, Garcia Costas AM,Overmann J, Ward DM (2012) Comparative and functional genomics of anoxygenic green bacteria from the taxa *Chlorobi,*

Chloroflexi, and *Acidobacteria.* In: Burnap RJ, Vermaas W (eds) Functional Genomics and Evolution of Photosynthetic Systems Springer, Dordrecht, The Netherlands, Vol. 33, pp 47–102
Brock TD (1978) Thermophilic microorganisms and life at high temperatures. Springer, Berlin, 465 pp
Castenholz RW (1968) The behavior of *Oscillatoria terebriformis* in hot springs. J Phycol 4:132–139
Castenholz RW (1969) Thermophilic blue-green algae and the thermal environment. Bacteriol Rev 33:476–504
Castenholz RW (1973a) Ecology of blue-green algae in hot springs. In: Carr NG, Whitton BA (eds) The biology of blue-green algae. Blackwell/University of California Press, Oxford/Berkeley, pp 379–414, 676 pp
Castenholz RW (1973b) The possible use of sulfide by the filamentous phototrophic bacteria of hot springs. Limnol Oceanogr 18:863–867
Castenholz RW (1976) The effect of sulfide on the blue-green algae of hot springs. I. New Zealand and Iceland. J Phycol 12:54–68
Castenholz RW (1977) The effect of sulfide on the blue-green algae of hot springs. II. Yellowstone National Park. Microb Ecol 3:79–105
Castenholz RW (1978) The biogeography of hot spring algae through enrichment cultures. Mitt Int Ver Limnol 21:296–315
Castenholz RW (1992) Species usage, concept, and evolution in the cyanobacteria (blue green algae). J Phycol 28:737–745
Castenholz RW (1996) Endemism and biodiversity of thermophilic cyanobacteria. Nova Hedwig Beih 112:33–47
Castenholz RW, Utkilen HC (1984) Physiology of sulfide tolerance in a thermophilic cyanobacterium. Arch Microbiol 138:306–309
Cohan FM, Perry EB (2007) A systematics for discovering the fundamental units of bacterial diversity. Curr Biol 17:R373–R386
Cohen Y, Jørgensen BB, Revsbeck NP, Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407
Ferris MJ, Ward DM (1997) Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. Appl Environ Microbiol 63:1375–1381
Ferris MJ, Ruff-Roberts AL, Kopczynski ED, Bateson MM, Ward DM (1996) Enrichment culture and microscopy conceal diverse thermophilic *Synechococcus* populations in a single hot spring microbial mat habitat. Appl Environ Microbiol 62:1045–1050
Ferris MJ, Nold SC, Revsbech NP, Ward DM (1997) Population structure and physiological changes within a hot spring microbial mat community following disturbance. Appl Environ Microbiol 63:1367–1374
Ferris MJ, Kühl M, Wieland A, Ward DM (2003) Different light-adapted ecotypes in a 68°C *Synechococcus* mat community revealed by analysis of 16S-23S intervening transcribed spacer variation. Appl Environ Microbiol 69:2893–2898
Finlay B (2002) Global dispersal of free-living microbial species. Science 296:1061–1063
Garcia-Pichel F, Castenholz RW (1990) Comparative anoxygenic photosynthetic capacity in 7 strains of a thermophilic cyanobacterium. Arch Microbiol 153:344–351
Giovannoni SJ, Revsbech NP, Ward DM, Castenholz RW (1987) Obligately phototrophic *Chloroflexus*: primary production in anaerobic hot spring microbial mats. Arch Microbiol 147:80–89
Hey J, Nielsen R (2004) Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. Genetics 167:747–760
Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294:2310–2314
Inskeep WP, Rusch DB, Jay ZJ, Herrgard MJ, Kozubal MA, Richardson TH, Macur RE, Hamamura N, RdeM J, Fouke BW, Reysenbach A-L, Roberto F, Young M, Schwartz A, Boyd ES, Badger JH, Mathur EJ, Ortmann AC, Bateson M, Geesey G, Frazier M (2010) Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. PLoS One 5:e9773
Ionescu D, Hindiyeh M, Malkawi H, Oren A (2010) Biogeography of thermophilic cyanobacteria: insights from the Zerka Ma'in hot springs (Jordan). FEMS Microbiol Ecol 72:103–113
Jensen SI, Steunou A-S, Bhaya D, Kühl M, Grossman AR (2010) *In situ* dynamics of O_2, pH and cyanobacterial transcripts associated with CCM, photosynthesis and detoxification of ROS. ISME J 5:317–328
Jing H, Aitchison JC, Lacap DC, Peerapornpisal Y, Sompong U, Pointing SB (2005) Community phylogenetic analysis of moderately thermophilic cyanobacterial mats from China, the Philippines and Thailand. Extremophiles 9:325–332
Jørgensen BB, Nelson DC (1988) Bacterial zonation, photosynthesis, and spectral light distribution in hot spring microbial mats of Iceland. Microb Ecol 16:133–147
Kallas T, Castenholz RW (1982a) Internal pH and ATP-ADP pools in the cyanobacterium *Synechococcus* sp. during exposure to growth-inhibiting low pH. J Bacteriol 149:229–236
Kallas T, Castenholz RW (1982b) Rapid transient growth at low pH in the cyanobacterium *Synechococcus* sp. J Bacteriol 149:237–246
Kilian O, Steunou A-S, Fazeli F, Bailey S, Bhaya D, Grossman AR (2007) Responses of a thermophilic *Synechococcus* isolate from the microbial mat of Octopus Spring to light. Appl Environ Microbiol 73:4268–4278
Klatt CG, Bryant DA, Ward DM (2007) Comparative genomics provides evidence for the 3-hydroxypropionate autotrophic pathway in filamentous anoxygenic phototrophic bacteria and in hot spring microbial mats. Environ Microbiol 9:2067–2078
Klatt CG, Liu Z, Ludwig M, Kuhl M, Jensen SI, Bryant DA, Ward DM (2012a) Temporal patterning of in situ gene expression in uncultivated phototrophic Chloroflexi inhabiting a microbial mat in an alkaline siliceous geothermal spring (in preparation)
Klatt, CG, Inskeep, WP, Herrgard, M, Rusch, DB, Tringe, SG, Jay, Z, Parenteau, MN, Ward, DM, Boomer, SM, Bryant, DA, Miller, SR. (2012b) Comparative analysis of community structure and function among phototrophic microbial mats inhabiting diverse geothermal environments (in preparation)
Klatt CG, Wood JM, Rusch DB, Bateson MM, Hamamura N, Heidelberg JF, Grossman AR, Bhaya D, Cohan FM, Kühl M, Bryant DA, Ward DM (2011) Community ecology of hot spring cyanobacterial mats: predominant populations and their functional potential. ISME J. 5:1262–1278
Koeppel A, Perry EB, Sikorski J, Krizanc D, Warner A, Ward DM, Rooney AP, Brambilla E, Connor N, Nevo E, Ratcliff RM, Cohan FM (2008) Identifying the fundamental units of bacterial diversity: a paradigm shift to incorporate ecological diversification into bacterial systematics. Proc Natl Acad Sci USA 105:2504–2509
Konopka A (1992) Accumulation and utilization of polysaccharide by hot-spring phototrophs during a light-dark transition. FEMS Microbiol Ecol 102:27–32
Külköylüoglu O, Meisch C, Rust RW (2003) *Thermopsis thermophila* n. gen. n. sp. from hot springs in Nevada (Crustacea, Ostracoda). Hydrobiologia 499:113–123
Lacap DC, Barraquio W, Pointing SB (2007) Thermophilic microbial mats in a tropical geothermal location display pronounced seasonal changes but appear resilient to stochastic disturbance. Environ Microbiol 9:3065–3076
Lau E, Nash CZ, Vogler DR, Cullings KW (2005) Molecular diversity of cyanobacteria inhabiting coniform structures and surrounding mat in a Yellowstone hot spring. Astrobiology 5:83–92
Lau CY, Aitchison JC, Pointing SB (2006) Highly diverse community structure in a remote central Tibetan geothermal spring does not display monotonic variation to thermal stress. FEMS Microbiol Ecol 57:80–91

Lau CY, Aitchison JC, Pointing SB (2008) Early colonization of thermal niches in a silica-depositing hot spring in central Tibet. Geobiology 6:136–146

Lau MCY, Aitchison JC, Pointing SB (2009) Bacterial community composition in thermophilic microbial mats from five hot springs in central Tibet. Extremophiles 13:139–149

Liu X, Klatt CG, Ludwig M, Rusch DB, Jensen SI, Kuhl M, Ward DM, Bryant DA (2012) "*Candidatus Thermochlorobacter aerophilum*:" an aerobic chlorophotoheterotrophic member of the phylum Chlorobi defined by metagenomics and metatranscriptomics. ISME J. (in press)

Liu Z, Klatt CG, Wood JM, Rusch DB, Ludwig M, Wittekindt N, Tomsho LP, Schuster SC, Ward DM, Bryant DA (2011) Metatranscriptomic analyses of chlorophototrophs of a hot-spring microbial mat. ISME J 5(8):1279–1290. doi:10.1038/ismej.2011.37

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen Y-J, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer MLI, Jarvie TP, Jirage KB, Kim J-B, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380

Mariner RH, Rapp JB, Willey LM, Presser TM (1974) The chemical composition and estimated minimum thermal reservoir temperatures of selected hot springs in Oregon. US Geological Survey open file report, Mar 1994, Menlo Park, CA, 27 pp

Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach A-L, Smith VH, Staley JT (2006) Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol 4:102–112

Meeks JC, Castenholz RW (1971) Growth and photosynthesis in an extreme thermophile, *Synechococcus lividus* (Cyanophyta). Arch Mikrobiol 78:25–41

Melendrez MC, Cohan FM, Ward DM (2012) Cultivation-independent multi-locus sequence analysis of the impact of recombination on *Synechococcus* species populations inhabiting a hot spring cyanobacterial mat (in preparation)

Melendrez MC, Lange RC, Cohan FM, Ward DM (2011) Influence of molecular resolution on sequence-based discovery of ecological diversity among *Synechococcus* populations in an alkaline siliceous hot spring microbial mat. Appl Environ Microbiol 77(4): 1359–1367

Miller SR (2007) Diversity of the cosmopolitan thermophile *Mastigocladus laminosis* at global, regional, and local scales. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 399–410

Miller SR (2010) Population genetics of cyanobacteria. In: Xu J (ed) Microbial population genetics. Caister Academic Press, Norfolk, pp 97–107

Miller SR, Castenholz RW (2000) Evolution of thermotolerance in hot spring cyanobacteria of the genus *Synechococcus*. Appl Environ Microbiol 66:4222–4229

Miller SR, Wingard CE, Castenholz RW (1998) Effects of visible light and UV radiation on photosynthesis in a population of a hot spring cyanobacterium, a *Synechococcus* sp., subjected to high-temperature stress. Appl Environ Microbiol 64:3893–3899

Miller SR, Purugganan MD, Curtis SE (2006) Molecular population genetics and phenotypic diversification of two populations of the thermophilic cyanobacterium *Mastigocladus laminosus*. Appl Environ Microbiol 72:2793–2800

Miller SR, Castenholz RW, Pedersen D (2007) Phylogeography of the thermophilic cyanobacterium *Mastigocladus laminosus*. Appl Environ Microbiol 73:4751–4759

Miller SR, Strong AL, Jones KL, Ungerer MC (2009a) Bar-coded pyrosequencing reveals shared bacterial community properties along the temperature gradients of two alkaline hot springs in Yellowstone National Park. Appl Environ Microbiol 75:4565–4572

Miller SR, Williams C, Strong AL, Carvey D (2009b) Ecological specialization in a spatially structured population of the thermophilic cyanobacterium *Mastigocladus laminosus*. Appl Environ Microbiol 75:729–734

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158:885–896

Nold SC, Ward DM (1996) Photosynthate partitioning and fermentation in hot spring microbial mat communities. Appl Environ Microbiol 62:4598–4607

Norris TB, McDermott TD, Castenholz RW (2002) The long term effects of UV exclusion on the microbial composition and photosynthetic competence of bacteria in hot spring microbial mats. FEMS Microbiol Ecol 39:193–209

Nübel U, Bateson MM, Vandieken V, Kühl M, Ward DM (2002) Microscopic examination of distribution and phenotypic properties of phylogenetically diverse *Chloroflexaceae*-related bacteria in hot spring microbial mats. Appl Environ Microbiol 68:4593–4603

Papke RT, Ramsing NB, Bateson MM, Ward DM (2003) Geographical isolation in hot spring cyanobacteria. Environ Microbiol 5:650–659

Peary JA, Castenholz RW (1964) Temperature strains of a thermophilic blue-green alga. Nature 202:720–721

Portillo MC, Sririn V, Kanoksilapatham W, Gonzalez JM (2009) Differential microbial communities in hot spring mats from Western Thailand. Extremophiles 13:321–331

Ramsing NB, Ferris MJ, Ward DM (2000) Highly ordered vertical structure of *Synechococcus* populations within the one-millimeter thick photic zone of a hot spring cyanobacterial mat. Appl Environ Microbiol 66:1038–1049

Revsbech NP, Ward DM (1984) Microelectrode studies of interstitial water chemistry and photosynthetic activity in a hot spring microbial mat. Appl Environ Microbiol 48:270–275

Roeselers G, Norris TB, Castenholz RW, Rysgaard S, Glud RN, Kühl M, Muyzer G (2007) Diversity of phototrophic bacteria in microbial mats from Arctic hot springs (Greenland). Environ Microbiol 9:26–38

Sandbeck KA, Ward DM (1981) Fate of immediate methane precursors in low sulfate hot spring algal-bacterial mats. Appl Environ Microbiol 41:775–782

Sompong U, Hawkins PR, Besley C, Peerapornpisal Y (2008) The distribution of cyanobacteria across physical and chemical gradients in hot springs in northern Thailand. FEMS Microbiol Ecol 52:365–376

Sorokovikova EG, Tikhonova IV, Belykh OI, Klimenkov IV, Likhoshwai EV (2008) Identification of two cyanobacterial strains isolated from the Kotel'nikovskii hot spring of the Baikal rift. Microbiology 77:365–372

Steunou A-S, Bhaya D, Bateson MM, Melendrez MC, Ward DM, Brecht E, Peters JW, Kühl M, Grossman AR (2006) In situ analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. Proc Natl Acad Sci USA 103:2398–2403

Steunou A-S, Jensen SI, Brecht E, Becraft ED, Bateson MM, Kilian O, Bhaya D, Ward DM, Peters JW, Grossman AR, Kühl M (2008) Regulation of *nif* gene expression and the energetics of N_2 fixation over the diel cycle in a hot spring microbial mat. ISME J 2:364–378

Stewart WDP (1970) Nitrogen fixation by blue-green algae in Yellowstone thermal areas. Phycologia 9:261–268

van der Meer MTJ, Schouten S, de Leeuw JW, Ward DM (2000) Autotrophy of green non-sulphur bacteria in hot spring microbial mats: biological explanations for isotopically heavy organic carbon in the geological record. Environ Microbiol 2:428–435

van der Meer MTJ, Schouten S, Sinninghe-Damsté JS, de Leeuw JW, Ward DM (2003) Compound-specific isotopic fractionation patterns suggest different carbon metabolisms among *Chloroflexus*-like bacteria in hot spring microbial mats. Appl Environ Microbiol 69:6000–6006

van der Meer MTJ, Schouten S, Bateson MM, Nübel U, Wieland A, Kühl M, de Leeuw JW, Sinninghe-Damsté JS, Ward DM (2005) Diel variations in carbon metabolism by green nonsulfur-like bacteria in alkaline silicious hot spring microbial mats from Yellowstone National Park, U.S.A. Appl Environ Microbiol 71:3978–3986

van der Meer MTJ, Schouten S, Sinninghe Damsté JS, Ward DM (2007) Impact of carbon metabolisms on ^{13}C signatures of cyanobacteria and green nonsulfur-like bacteria inhabiting a microbial mat from an alkaline siliceous hot spring in Yellowstone National Park (USA). Environ Microbiol 9:482–491

van der Meer MTJ, Klatt CG, Wood J, Bryant DA, Bateson MM, Lammerts L, Schouten S, Sinninghe Damsté JS, Madigan MT, Ward DM (2010) Cultivation and genomic, nutritional, and lipid biomarker characterization of *Roseiflexus* strains closely related to predominant *in situ* populations inhabiting Yellowstone hot spring microbial mats. J Bacteriol 192:3033–3042

Van Valen L (1976) Ecological species, multispecies, and oaks. Taxon 25:233–239

Vanyo JP, Hutchinson RA, Awramik SM (1986) Heliotropism in microbial stromatolitic growths at Yellowstone National Park: geophysical inferences. EOS 67:153–156

Walter MR, Bauld J, Brock TD (1972) Siliceous algal and bacterial stromatolites in hot spring and geyser effluents of Yellowstone National Park. Science 178:402–405

Ward DM (1998) A natural species concept for prokaryotes. Curr Opin Microbiol 1:271–277

Ward DM, Castenholz RW (2000) Cyanobacteria in geothermal habitats. In: Potts M, Whitton BA (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 37–59, 669 pp

Ward DM, Cohan FM (2005) Microbial diversity in hot spring cyanobacterial mats: pattern and prediction. In: Inskeep B, McDermott T (eds) Geothermal biology and geochemistry in Yellowstone National Park. Thermal Biology Institute, Montana State University, Bozeman, pp 185–201

Ward DM, Tayne TA, Anderson KL, Bateson MM (1987) Community structure, and interactions among community members in hot spring cyanobacterial mats. Symp Soc Gen Microbiol 41:179–210

Ward DM, Bateson MM, Ferris MJ, Nold SC (1998) A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. Microbiol Mol Biol Rev 62:1353–1370

Ward DM, Bateson MM, Ferris MJ, Kühl M, Wieland A, Koeppel A, Cohan FM (2006) Cyanobacterial ecotypes in the microbial mat community of Mushroom Spring (Yellowstone National Park, Wyoming) as species-like units linking microbial community composition, structure and function. Philos Trans R Soc Lond B 361:1997–2008

Ward DM, Cohan FM, Bhaya D, Heidelberg JF, Kühl M, Grossman A (2008) Genomics, environmental genomics and the issue of microbial species. Heredity 100:207–219

Ward DM, Klatt CG, Wood J, Cohan FM, Bryant DA (2012) Functional genomics in an ecological and evolutionary context: maximizing the value of genomes in systems biology. In: Burnap RL, Vermaas W (eds) Functional genomics and evolution of photosynthetic systems, vol 33. Springer, Dordrecht, pp. 1–16

Ward DM, Melendrez MC, Becraft ED, Klatt CG, Wood J, Cohan FM (2011) Metagenomic approaches for the identification of microbial species. In: de Bruijn FJ (ed) Handbook of molecular microbial ecology I: metagenomics and complementary approaches. Wiley, Hoboken, pp. 105–109

Whitaker RJ, Grogan DW, Taylor JW (2003) Geographic barriers isolate endemic populations of hyperthermophilic Archaea. Science 301:976–978

Wickstrom CE (1980) Distribution and physiological determinants of blue-green algal nitrogen fixation along a thermogradient. J Phycol 16:436–443

Wickstrom CE, Castenholz RW (1973) Thermophilic ostracod: aquatic metazoan with highest known temperature tolerance. Science 181:1063–1064

Wickstrom CE, Castenholz RW (1978) Association of *Pleurocapsa* and *Calothrix* (Cyanophyta) in a thermal stream. J Phycol 14:84–88

Wickstrom CE, Castenholz RW (1985) Dynamics of cyanobacterial and ostracod interactions in an Oregon hot spring. Ecology 66:1024–1041

Wright S (1943) Isolation by distance. Genetics 28:114–138

Yim LC, Hongmei J, Aitchison JC, Pointing SB (2006) Highly diverse community structure in a remote central Tibetan geothermal spring does not display monotonic variation to thermal stress. FEMS Microbiol Ecol 57:80–91

Yun Z (1986) Thermophilic microorganisms in the hot springs of Tengchong geothermal area, West Yunnan, China. Geothermics 15:347–358

Cyanobacterial Mats and Stromatolites

4

Lucas J. Stal

Contents

L.J. Stal (✉)
Department of Marine Microbiology, Royal Netherlands Institute of Sea Research (NIOZ),
P. O. Box 140, NL-4400 AC Yerseke, The Netherlands
e-mail: lucas.stal@nioz.nl

Summary

Cyanobacteria are often the key organisms comprising microbial mats. They form dense micrometer-scale communities in which the full plethora of microbial metabolism can be present. Such mats are therefore excellent model systems and because of their analogy with Precambrian stromatolites they are also attractive subjects for evolutionary studies. Growth and metabolism of the oxygenic phototrophic cyanobacteria enrich the sediment with organic matter. However, in mature mats net growth of cyanobacteria appears to be of less importance. Most of the organic matter produced from photosynthetic CO_2 fixation is liberated in the sediment by one of the following: fermentation, photorespiration, pouring out of solutes or secretion of mucus although grazing may also be important. This organic matter is degraded by chemotrophic microorganisms, among which sulphate-reducing bacteria are particularly prominent. The combined activities of the cyanobacteria and sulphate-reducing bacteria result in steep and fluctuating gradients of sulphide and oxygen. Cyanobacteria therefore have to cope with

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high concentrations of sulphide, oxygen supersaturated – and anoxic conditions. These physicochemical gradients force different functional groups of microorganisms to particular vertical stratified positions in the mat. This, and the fact that accretion of sediment fluctuates, gives rise to one of the most conspicuous properties of microbial mats namely their laminated structure. Modern microbial mats have this laminated structure in common with Precambrian stromatolites. Most modern mats do not lithify but this may also have been the case for Archean microbial mats. Only a few examples of modern calcifying stromatolithic microbial mats are known. A hypothesis has been developed which conceives a role for extracellular polysaccharides in calcification. Extracellular polysaccharides in cyanobacterial mats are often produced as the result of unbalanced growth caused by nitrogen deficiency. The mat organisms are embedded in the extensive polysaccharide matrix that inhibits calcification. All cyanobacterial mats can fix atmospheric dinitrogen, which covers part of their nitrogen demand, but the fluctuating physicochemical gradients limits the efficiency of this process.

4.1 Introduction

The term microbial mat is used for multilayered microbial communities growing on sediments in diverse habitats such as tidal sand flats, hypersaline ponds, hot springs and other. Microbial mats are generally formed by filamentous, entangled organisms that produce a macroscopic 'mat-like' structure. In some cases such mats can indeed be peeled off from the sediment as a large coherent piece. However, benthic microbial communities of unicellular organisms, that usually do not form such coherent structures, are also called mats. Microbial mats exhibit great variety in morphology and composition, and they may include mats of diatoms and other biofilms of immobilized microorganisms (Bauld 1984). Nevertheless, eukarya are few or excluded altogether from environments in which extreme conditions prevail but in some cases meiofauna and other grazers are active in the habitats in which microbial mats are formed. One reason for the exclusion of eukarya is the wide spectrum of metabolic capabilities of bacteria and archaea and the great capacity these 'prokaryotes' display to adapt to changes and fluctuations in environmental conditions. Purple and sometimes green sulphur bacteria are normal components of most cyanobacterial mats (Nicholson et al. 1987; Pierson et al. 1987). This review focuses on mats formed by cyanobacteria.

The reason why cyanobacteria are typically the most successful mat-building organisms may be found in the combination of a number of the characteristic properties of this unique group of microorganisms. Cyanobacteria are the only oxygenic phototrophic bacteria and this metabolism is absent in archaea. As their predominant metabolism is oxygenic photosynthesis, cyanobacteria use light as an energy source, water as an electron donor and CO_2 as a carbon source. These are the major requirements for growth and are abundant in the environments where most microbial mats are found. Another important property of many cyanobacteria, which is not shared by eukarya (and hence not by algae), is their ability to fix atmospheric N_2, allowing them to grow independent of a source of combined nitrogen. Photosynthesis in cyanobacteria saturates at low light intensity, cyanobacteria have a high affinity for light, and maintenance requirements are extremely low (Van Liere and Mur 1979). These properties allow photosynthesis even under extremely low light conditions. Moreover, several species are capable of sulphide-dependent anoxygenic photosynthesis (Garlick et al. 1977). Mat-forming cyanobacteria are well-adapted for life under anoxic conditions. In addition to the normal aerobic dark respiration, virtually all species of cyanobacteria in microbial mats are capable of fermentation (Stal and Moezelaar 1997). These properties of cyanobacteria are essential for life in microbial mats in which environmental conditions strongly fluctuate.

A typical property of microbial mats is their laminated structure in which different functional groups of microorganisms occur in vertically stratified layers (Stal et al. 1985). In addition to biological stratification biomineralogical stratification can be distinguished (Monty 1976). This type of lamination can be attributed to different growth periods, seasonal events, periodical events (e.g. tides) or episodic or erratic events (e.g. storms). Often, this laminated pattern is restricted, since most of the organic matter of the mat is degraded. When conditions allow, mats precipitate minerals, mainly calcite (Golubić 1973; Monty 1976; Krumbein 1979). This calcification is strongly associated with microbial metabolism and it may therefore give rise to the formation of distinct laminae and eventually to consolidated rock. Laminated rocks dating from the Precambrian and later eras are known as stromatolites (Krumbein 1983). Modern microbial mats built by cyanobacteria show remarkable similarities to fossil stromatolites. Stromatolites date back to 3.5 billion years (Mason and Von Brunn 1977; Lowe 1980; Walter et al. 1980; Orpen and Wilson 1981; Chap. 2). In some of these stromatolites well-preserved microfossils have been found that in some cases showed a remarkable resemblance to modern cyanobacteria (Schopf and Walter 1982; Awramik 1984; Chap. 2). It is therefore attractive to consider modern microbial mats as analogues of Precambrian stromatolites; however, structural differences do not always seem to justify this comparison. A major problem is the fact that the great majority of present day microbial mats does not form consolidated rock.

This review will discuss the metabolic activities of cyanobacteria that allow them to form microbial mats and stromatolites. This is a revised, updated and extended version of the chapter with the same title that appeared in the first edition of The Ecology of Cyanobacteria (Stal 2000).

4.2 Microbial Mats, Stromatolites and Their Environments

4.2.1 What Are Microbial Mats and Stromatolites? Some Definitions

Krumbein (1983), referring to the work of Kalkowsky (1908), proposed the following definition: "*Stromatolites are laminated rocks, the origin of which can clearly be related to the activity of microbial communities, which by their morphology, physiology, and arrangement in space and time interact with the physical and chemical environment to produce a laminated pattern which is retained in the final rock structure*". This definition includes fossil as well as recent formations. Modern stromatolites that fit this definition are rare. Awramik and Margulis (in Walter 1976) defined stromatolites as: "*Organosedimentary structures produced by sediment trapping, binding and/or precipitation as a result of the growth and metabolic activity of microorganisms, principally cyanophytes*". This definition includes fossil and recent consolidated stromatolites as well as unconsolidated microbial mats. Both definitions, however, emphasize the role of microbial mats and their microflora in the formation of stromatolites. Walter (1976) formulated the following conditions necessary to form a microbial mat:

(i) environmental conditions must allow growth of the mat-building microorganisms; growth rate of the mat-building organisms must be faster than consumption by grazers;
(ii) sedimentation rates should not be exceedingly high to allow stabilized colonization of the surface by the mat-building organisms;
(iii) destructive forces from burrowing organisms and mechanical and chemical erosion must be absent or at least not prevent accretion of organisms.

In order to produce a stromatolite, preservation of the structure must occur. In modern day environments unconsolidated microbial mats are formed, i.e. systems that do not have the potential to preserve its structure, defined by Krumbein (1983) as: "*Unconsolidated laminated systems, clearly related to the activity of microbial communities, often called recent stromatolites or living stromatolites are defined as potential stromatolites*". Indeed, stromatolites *sensu* Krumbein are still being formed today. Excellent examples of consolidated, well-laminated stromatolites formed by the growth and metabolic activity of a microbial mat are found in the Exuma Cays, Bahamas (Reid and Browne 1991; Pinckney et al. 1995). Stromatolites are just one form of calcified microbial mats that are jointly termed microbialites, a term that includes thrombolites, characterized by a cohesive macrofabric, and leiolites, which are without defined structure (Dupraz et al. 2009).

Also, non-consolidated, non-lithified microbial mats may leave traces of microbially induced sedimentary structures especially in siliciclastic deposits in shallow coastal environments (abbreviated as MISS) (Noffke et al. 2006). These structures have in some cases been preserved in the fossil record going back to the early Archean, emphasizing that not all Archean microbial mats were microbialites.

Since their discovery in 1961 in Shark Bay, Western Australia, poorly-laminated consolidated calcareous stromatolites (thrombolites) have been presented as strikingly similar to Precambrian stromatolites (Logan 1961). Contemporary calcareous stromatolites are also formed in Polynesian atolls (Kopara) (Défarge et al. 1994a, b) and other lacustrine and perimarine settings (Kempe et al. 1991; Kempe and Kazmierczak 1993). Such stromatolites can be called 'modern stromatolites' to distinguish them from fossil formations. It is not necessary to name unconsolidated microbial mats 'potential stromatolites', since they are not stromatolites (*sensu* Krumbein) and most of these microbial mats will never become such. Nevertheless, unconsolidated mats may keep in themselves the capacity for consolidation. This was shown by a transplantation experiment in which a non-lithifying microbial mat was placed in an environment with lithifying microbial mats. This mat calcified, demonstrating its potential to become a microbialite. Calcification clearly depends on the prevailing local environmental conditions (Dupraz et al. 2009).

Calcification is required for consolidation and preservation of microbial mats. In many microbial mats, calcification does not occur and the reasons for this are discussed later on in this chapter. Many consolidated rocks without a clear lamination are formed by microbial communities. It may be that laminations were lost during the process of diagenesis or that neither vertical stratified communities of microorganisms nor clear seasonal variations were involved in growth and metabolic activity. Another proposed mechanism is that carbonate sand accretes through trapping and binding in the microbial mat without *in situ* calcification. Such cohesive but poorly laminated microbialites are known as thrombolites (Kennard and James 1986). The stromatolites of the Exuma Cays, Bahamas, in contrast with other recent formations, possess a fine laminated structure. There, in addition to trapping and binding of carbonate sand, *in situ* precipitation of calcium carbonate produces distinct layers of cement (Reid and Browne 1991).

4.2.2 Microbial Mats and Stromatolites: The Geological Evidence

The Hadean era from the origin of the Earth 4.5×10^9 years before present to 3.9×10^9 is the period of which no rock record exists. The oldest rock known from the early Archean

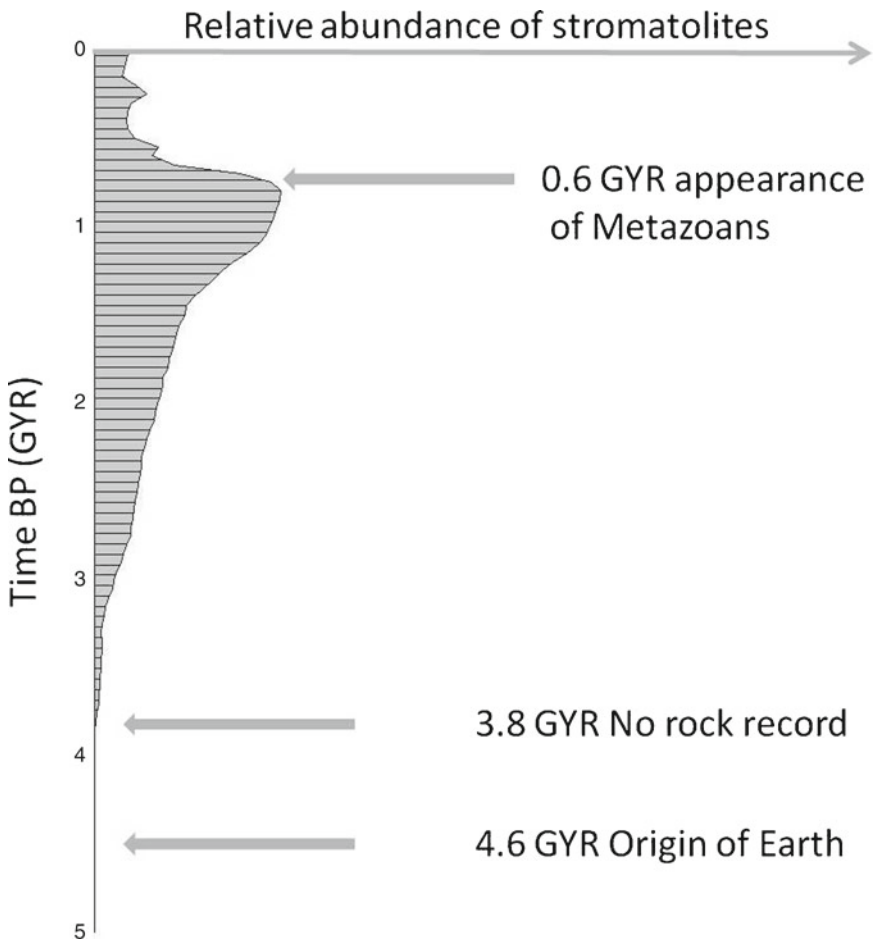

Fig. 4.1 Relative abundance of stromatolites plotted against time (After Awramik 1984)

may not be of biogenic origin. The oldest stromatolites date back in the Archean about 3.5×10^9 years ago. Only a few examples are known from this era, but they were undoubtedly biologically produced. Microfossils have been found in these stromatolites, but it is premature to identify them as cyanobacteria. From measurements of carbon isotope compositions in these rocks it was deduced that autotrophic microorganisms must have been active at that time. However, it could have been chemoautotrophs that fixed the CO_2, rather than photoautotrophs. The morphology of the microfossils from these oldest stromatolites also does not give an unequivocal clue about the identity of the organisms. Cyanobacteria are a group of oxygenic phototrophic organisms and it is well established that the Archean atmosphere did not contain oxygen.

During the Proterozoic, which started about 2.5×10^9 years ago, stromatolites became abundant (Fig. 4.1) and occur in a wide variety of facies. They occupied every major ecological niche, marine and lacustrine, shallow and deep water. Most of limestones, dolomites and magnesites as well as many phosphorites and iron formations of the Proterozoic contain stromatolites. Like modern microbial mats, it seems certain that the Proterozoic stromatolites were produced by growth and metabolic activity of cyanobacteria. The Proterozoic stromatolites contain a wealth of very well preserved microfossils that show striking similarity to present day cyanobacteria. Over 1,100 microfossils have been described from 190 stromatolite formations (Walter et al. 1992). Many of these fossils are preserved in the cherts of stromatolites. The best preservation occurred following early silicification of the stromatolites. Silica precipitation occurred possibly spontaneously because of its supposed high concentration in the seawater. Diatoms with their silicate frustules had not evolved yet and no other sink for silica is known.

Oxygen was present in the atmosphere at 2.3×10^9 years before present. It is now well accepted that the oxygenation of the atmosphere was the result of oxygenic photosynthesis. It might have taken considerable time after the origin of oxygenic photosynthesis until the atmosphere became oxygenated, since a large amount of reduced compounds had to be oxidized. Banded iron formations (BIFs) are known from 2.5×10^9 years before present. These are huge formations consisting of oxidized iron and they have been taken as evidence for the presence of oxygenic photosynthesis. However, iron oxidation could also have taken place by the activity of anoxygenic phototrophic bacteria under anaerobic conditions

(Widdel et al. 1993; Ehrenreich and Widdel 1994) or perhaps even by iron-dependent anoxygenic photosynthesis by cyanobacteria (Cohen 1989). Oxygenic photosynthesis most likely evolved at the beginning of the Proterozoic. Evidence for this is the presence of molecular markers such as the methylhopanes that are supposed to be specific for cyanobacteria 2.7×10^9 years before present (Brocks et al. 1999). Also, phylogenetic analysis date the origin of cyanobacteria at 2.6×10^9 (Hedges et al. 2001). However, many cyanobacteria in present day microbial mats are capable of anoxygenic photosynthesis and it seems likely that cyanobacteria were anoxygenic phototrophs before they evolved oxygenic photosynthesis (Olson 2006).

The morphology of the stromatolites of the 3.1×10^9 old Insuzi group of South Africa hints at the involvement in their formation of tactic filamentous organisms (Mason and Von Brunn 1977). Although it is tempting to suspect phototaxis and hence potentially photosynthetic organisms, a chemotactic response would also explain the structure of this formation (Schopf and Walter 1982).

Of the many different morphological forms of microfossils, some can be traced back to present day cyanobacteria such as *Oscillatoria* and *Lyngbya* (Schopf and Walter 1982). These organisms are common in modern microbial mats where they may be involved in N_2 fixation. It is difficult to determine whether these ancient mats were diazotrophic, although the signature of the stable isotope ^{15}N might give some hints in the direction of diazotrophic (N_2-fixing) cyanobacteria (Bauersachs et al. 2009). Microfossils resembling cyanobacteria of the heterocystous genera *Nostoc* and *Scytonema* were abundant in Archean stromatolites (Schopf and Walter 1982), and this is taken as evidence that N_2 fixation might have been important. No remnants of heterocysts are known, probably because these cells did not fossilize well. Fossil remnants of akinetes which are survival stages of cells that are known only from heterocystous cyanobacteria are known dating back 1.5×10^9 years (Srivastava 2005). However, they may not have originated from microbial mats and it is unknown whether they were already associated with heterocystous cyanobacteria. To date, heterocystous cyanobacteria are uncommon in most microbial mats.

Proterozoic stromatolites reached maxima in numbers and diversity towards the end of this era, after which it showed a rapid decline (Walter and Heys 1985) (Fig. 4.1). It has been postulated that metazoa, which then appeared on Earth, were responsible for this decline (Walter and Heys 1985). The grazing activity of these animals would prevent the accumulation of the benthic mat-forming organisms and destroyed the fabric of microbial mats by bioturbation. After the appearance of metazoa, microbial mats would be much more limited in their distribution and developed in environments in which these grazers are largely excluded (so-called extreme environments). Nevertheless, based on evidence from a modern hypersaline lagoon in Venezuela, Gingras et al. (2011) have suggested that the early evolution of mobile complex animals may have been in cyanobacterial dominated mats during the Ediacaran period (635–542 million years ago).

The appearance of algae that competed successfully for light and nutrients in many environments could help explain the eventual pushing back of cyanobacterial mats to extreme environments. Also, sea level changes, caused by changes of climate and by tectonic processes, could explain the sudden decrease in stromatolite abundance (Gebelein 1976). And finally, the seawater in the Proterozoic might have been greatly oversaturated with respect to calcium carbonate (alkaline soda ocean) facilitating the calcification and preservation of stromatolites, which is less the case in the modern moderately alkaline ocean (Kempe and Kazmierczak 1990a).

Proterozoic stromatolites probably formed through one or more of the following (Walter et al. 1992):

(i) *in situ* precipitation as cement;
(ii) *in situ* precipitation as micrite either accreted passively from suspension or through trapping and binding of the grains by the mat microorganisms;
(iii) precipitation of micrite imported from adjacent environments.

The fine and distinct lamination of Proterozoic stromatolites hints at *in situ* precipitation. Most Phanerozoic stromatolites are probably produced by trapping and binding of carbonate and sand grains and therefore show poor or no lamination (Cloud and Semikhatov 1969).

4.2.3 Stratification and Structure of Microbial Mats and Stromatolites

Microbial mats are characterized by the vertical stratification of different functional groups of microorganisms. This structure is the result of the physicochemical gradients that are present in mats and in fact produced by the metabolic activity of the mat organisms themselves (Jørgensen et al. 1983). The typical structure of a microbial mat build by cyanobacteria is schematically depicted in Fig. 4.2.

Cyanobacteria evidently form the top layer of microbial mats although they are sometimes overlain by a film of diatoms. These organisms need to harvest light for photosynthesis and are essentially aerobic organisms. The cyanobacteria may further be covered by a layer of sand or sediment of varying thickness or be covered by an organic-rich mucilaginous layer which may contain photoprotective pigments such as scytonemin, which is produced by cyanobacteria. It occurs predominantly in the extracellular polysaccharide sheaths. Scytonemin is highly recalcitrant remaining in the empty sheaths that are left behind by the cyanobacteria. Scytonemin protects the underlying community from damage

caused by UV irradiation (sunglass effect) (Garcia-Pichel and Castenholz 1991; Chap. 19). The organic matter introduced in the sediment through the photosynthetic activity of the cyanobacteria is decomposed by a variety of chemotrophic microorganisms. The degradation of organic matter and the accompanying demand of oxygen result in permanent anoxic conditions below the layer of cyanobacteria (Fig. 4.3). Obligate anaerobic sulphate-reducing bacteria play a major role in the decomposition of organic material in marine cyanobacterial mats and other sulphide dominated environments. These bacteria produce sulphide, which is used by anoxygenic phototrophic bacteria.

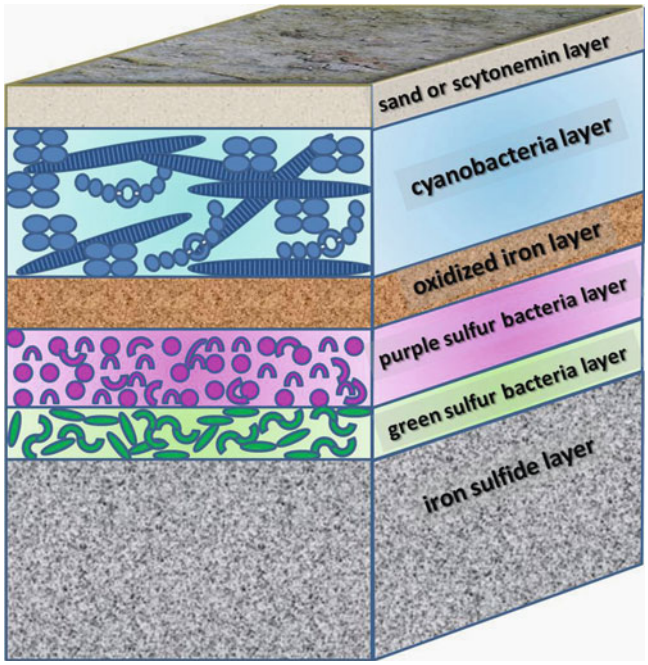

Fig. 4.2 Schematic drawing of a building of a typical microbial mat formed by cyanobacteria. The layer of green sulphur bacteria has been observed in only a few occasions

Purple sulphur bacteria are very common in microbial mats and are often seen as a pink layer below the cyanobacteria. Purple sulphur bacteria are essentially anaerobic bacteria, but species that occur in microbial mats are usually metabolically versatile (van Gemerden 1993). Anoxygenic photosynthesis in purple sulphur bacteria saturates at even much lower light intensities than photosynthesis in cyanobacteria. In addition, these organisms use a different part of the electromagnetic spectrum, not used by cyanobacteria (Pierson et al. 1987). This far red light is also least attenuated by the sediment (Fig. 4.4) (Stal et al. 1985; Jørgensen and Des Marais 1988). The biological stratification is thus the result of gradients of light, oxygen and sulphide and is found in virtually all cyanobacterial mats (Fig. 4.3). In some rare cases a layer of green anoxygenic phototrophic bacteria can be found underneath the purple bacteria (Nicholson et al. 1987).

A distinct layer of oxidized iron may be present between the cyanobacteria and the purple sulphur bacteria (Stal 1994). It is not clear how this layer is formed. It may be formed by chemical oxidation by the oxygen produced during photosynthesis. An alternative explanation is the anaerobic oxidation of iron by anoxygenic photosynthesis in a specific group of purple bacteria (Widdel et al. 1993; Ehrenreich and Widdel 1994). Aerobic oxidation of iron by chemotrophic bacteria seems unlikely at the alkaline pH that are usually encountered

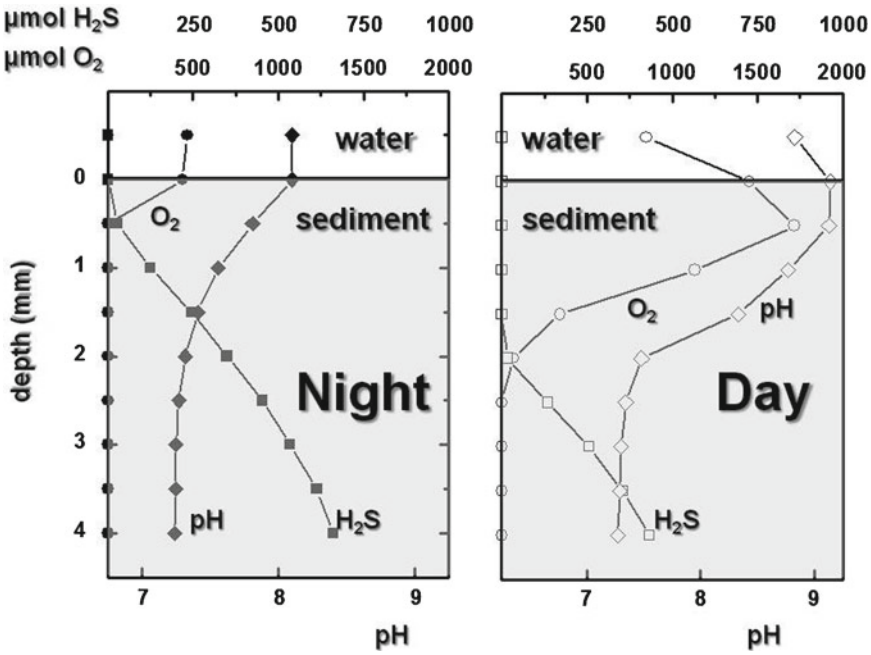

Fig. 4.3 Vertical profiles of oxygen, sulphide and pH at night (*left panel*) and during the day (*right panel*) in a mat of *Microcoleus chthonoplastes* from Solar Lake, Sinai (Redrawn from Revsbech et al. 1983)

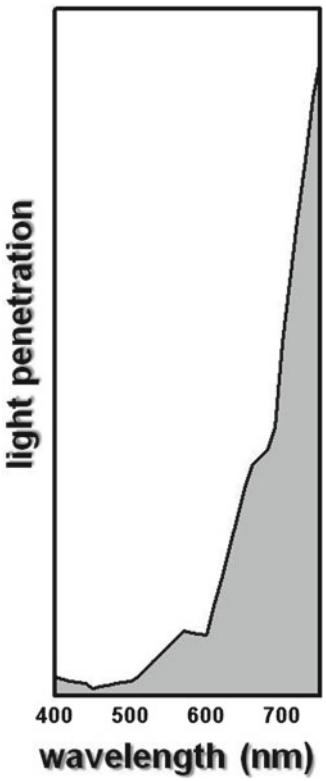

Fig. 4.4 Spectral light penetration through a 1.5-mm mat of *Microcoleus chthonoplastes* of the North Sea island of Mellum (Germany). Far-red light is least attenuated by the mat. The light absorption by chlorophyll *a* and phycobiliproteins at respectively 680 and 600 nm is clearly visible

in microbial mats (Fig. 4.3) although it should also not be excluded as a possibility as shown by Emerson and Revsbech (1994a, b). Other forms of anoxygenic photosynthesis that could potentially be important are those using nitrite (Griffin et al. 2007) and arsenate (Budinoff and Hollibaugh 2008; Kulp et al. 2008), although expected only in special cases.

The deeper layer of the mat is often black or gray, indicating the presence of iron sulphide (FeS) or pyrite (FeS_2). This layer has often been referred to as the layer of the sulphate-reducing bacteria but it has become clear that these bacteria in fact do not form a distinct layer and occur throughout the sediment (Visscher et al. 1992; Stal 1993). They are both abundant and highly active in the top layers of microbial mats. At first sight this distribution of sulphate-reducing bacteria is unexpected and odd. However, their substrates are mainly produced by the cyanobacteria and it is certainly beneficial to the organisms to be close to the site of production. At night when photosynthesis ceases, the mat turns anoxic (Fig. 4.3), providing the appropriate environment for sulphate-reducing bacteria. Sulphate reducing bacteria appear to be quite tolerant to oxygen and some are even capable of low rates of aerobic respiration although they are unable to grow aerobically (Dilling and Cypionka 1990; Marschall et al. 1993). It has also been shown that sulphate reduction in a microbial mat can occur in the presence of oxygen (Canfield and Des Marais 1991; Fründ and Cohen 1992).

Chemotropic bacteria can also oxidize sulphide and represent an important group of organisms in microbial mats. As many as 2×10^9 cm^{-3} sediment of colourless sulphur bacteria has been detected in the top layer of microbial mats (Visscher et al. 1992). These bacteria are quantitatively important in microbial mats. Colourless sulphur bacteria are essentially aerobic and gain energy from the aerobic oxidation of sulphide. They are autotrophic organisms and fix CO_2 through the reductive pentose phosphate pathway (Calvin-Benson-Bassham cycle). Colourless sulphur bacteria have high affinities for their substrates and their presence cause highly dynamic oxygen and sulphide gradients, thereby overruling the chemical oxidation of sulphide. Since the sulphide-oxygen interface is highly dynamic and not fixed at a certain depth in the sediment (Fig. 4.3), these bacteria also do not form a distinct layer, although they are clearly most abundant in the top layer (Visscher et al. 1992). The joint metabolic activity of microorganisms in microbial mats results in steep physicochemical gradients of e.g. light, oxygen, sulphide, carbon dioxide and pH; these gradients shift markedly during a 24-h cycle (Fig. 4.3) (Jørgensen et al. 1979; Revsbech et al. 1983) and also respond to fluctuations of incident light. All microorganisms in microbial mats must therefore be highly versatile and flexible in order to respond to the continuous changes in environmental conditions.

The biological stratification in microbial mats may however be far more complex than described above. Cyanobacteria may be sandwiched between layers of anoxygenic phototrophic bacteria and even perform oxygenic photosynthesis there. Microbial mats may be also just 'inverted', with cyanobacteria occurring underneath the layer of anoxygenic phototrophic bacteria (Van Gemerden et al. 1989). This type of microbial mats can develop when much organic matter is deposited on the sediment and its degradation results in very high rates of sulphide production.

The construction of extensive clone libraries has shed new light on the structure and composition of the microbial mat community. While microscopic examination of the mat suggests that the cyanobacteria are the dominant component, clone libraries of the 16S rRNA gene tell a different story. Ley et al. (2006) found cyanobacteria only important in the top most layer of a microbial mat but most of the 16S rRNA sequences belonged to other bacteria. These authors found 752 species in 42 bacterial phyla and *Chloroflexi* were identified as the dominant organism both in terms of biomass and in numbers of 16S rRNA genes and were present throughout the mat. Most cyanobacteria are large microorganisms. They have only few copies of the 16S rRNA gene and their DNA is often difficult to extract from natural environments and to amplify by PCR. Even so, the importance of the diversity

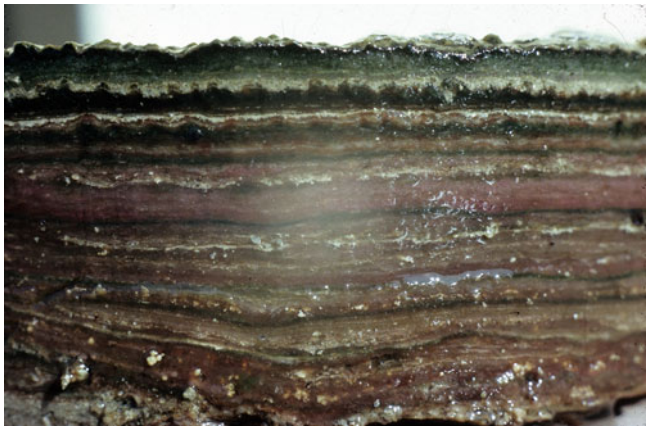

Fig. 4.5 The mat of *Microcoleus chthonoplastes* of hypersaline pond 5 of the saltern of Exportadora de Sal, S.A., Guerrero Negro, Baja California Sur, Mexico, showing the typical multilaminated structure

and biomass of other bacteria in microbial mats is probably severely underestimated. For instance, the clone libraries of the microbial mats of Hamelin Pool, Shark Bay, Western Australia, contained only less than 5% cyanobacteria. Ninety percent were bacteria and ten percent belonged to archaea. These mats did not reveal sequences belonging to eukarya (Papineau et al. 2005). The average sequence identity was only 92% emphasizing the high diversity of these microbial mats.

The organic matter produced by photosynthesis is actively cycled in mats. In mats net growth is often close to nil (Nold and Ward 1996). When growth occurs seasonally, a new mat may grow on top of the old one, resulting in a 'historical' lamination (Monty 1976). In many coastal environments the organic matter is fully degraded and a historical lamination is then absent. However, in other mats, particularly those growing in hypersaline environments, degradation may be incomplete. Examples of such mats are the well-investigated hypersaline mats of 'pond 5' in Guerrero Negro, Baja California, Mexico and those of Solar Lake, Sinai, Egypt (D'Antoni D'Amelio et al. 1989). The Guerrero Negro 'pond 5' mats are about 5–6 cm thick and are well-laminated (Fig. 4.5). This mat grows at a rate of about 1 cm year^{-1} but there is no net accretion, so that the thickness remains about the same. This means that the microbial decomposition of the mat also must proceed at a rate of approximately 1 cm year^{-1}. This mat therefore seems to be in steady state (Canfield and Des Marais 1994). The mineralization of the mat of Solar Lake is incomplete, although up to 99% of the primary production is immediately recycled in the mat, leaving only 1% for net accretion (Jørgensen and Cohen 1977; Krumbein et al. 1977). The Solar Lake microbial mat is about 1 m thick and the lamination goes back almost 2,000 years.

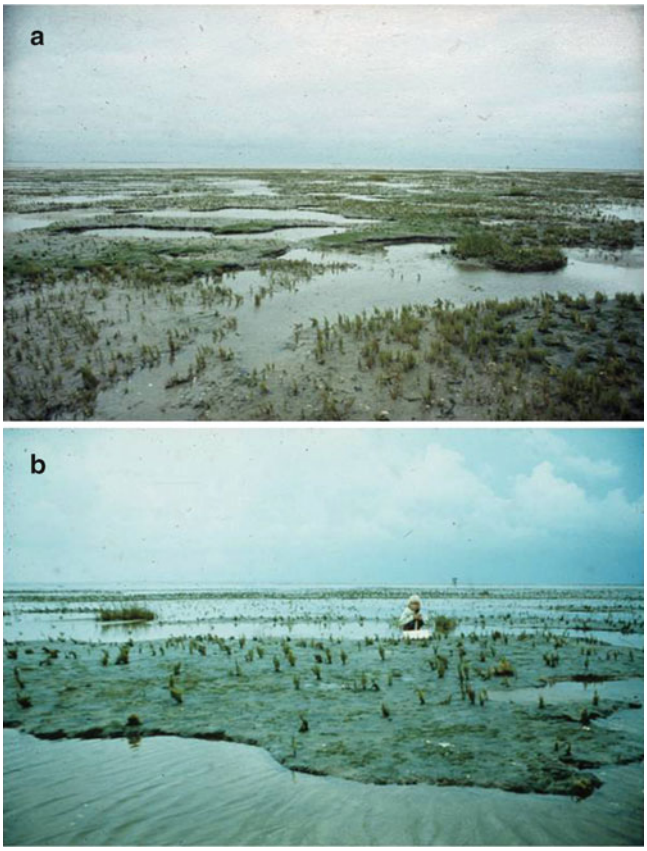

Fig. 4.6 (**a**) Extended tidal sand flat of the island of Mellum (Southern North Sea, Germany) at low tide covered with microbial mats. (**b**) Mature mats of *Microcoleus chthonoplastes* of the island of Mellum have accreted and fixed much sediment so that they are often not submersed at high tide. This decreases the grazing pressure

4.2.4 Environments Supporting Cyanobacterial Mats

4.2.4.1 Coastal Microbial Mats

Coastal tidal sand flats often are excellent environments for microbial mats to develop, particularly when the flats extend over a large area and when the slope of the flat is low (Fig. 4.6). Large areas will be covered by water for only a short period during the tide and often the sediment is not inundated for several days during neap tides. Such sediments often experience large fluctuations in water content, salinity and temperature, resulting in extreme conditions that limit the range of organisms able to inhabit this environment. The near absence of grazing organisms allows mat-building cyanobacteria and diatoms to accumulate. Coastal sand flats are usually nutrient-poor, but the phototrophic cyanobacteria have low nutrient demands and they can fix N_2. Moreover, most cyanobacteria resist long periods of drought, tolerating large fluctuations of salinity and temperature.

Often these coastal microbial mats are composed of filamentous cyanobacteria that form a dense entangled mass which traps and binds sediment particles. Such mats are clearly visible to the naked eye as massive structures that to a large extent resist erosion (Fig. 4.6b). Their sediment stabilizing effect is of great importance for coastal morphogenesis. Typical examples are found in tidal sand flats of islands of the southern North Sea (e.g. Mellum, Germany), along the east coast of North America (e.g. Great Sippewisset Marsh, Cape Cod, Massachusetts; Bird Shoal, North Carolina), Pacific Coast (e.g. Guerrero Negro, Mexico) (Fig. 4.7), Shark Bay and Spencer Gulf in, respectively, Western and South Australia. A more complete list is given by Pierson (1992). Most such coastal microbial mats are not stromatolites, but examples of stromatolites in coastal sediments can be found in El Hamira Bay, Sinai, where stromatolitic beachrock is formed (Krumbein 1979) and along the Exuma Cays of the Bahamas intertidal and subtidal stromatolites are formed (Reid and Browne 1991) (Fig. 4.8). *Schizothrix* sp. can settle there in spite of the high wave energy to which the Atlantic coast of the Bahamas is exposed. Calcification of the Exuma Cays microbial mats renders stability to the system, which is necessary to cope with the strong wave energy.

Other coastal mats are present in protected lagoons and are semi-permanently inundated. Examples of such coastal lagoons in which benthic microbial mats develop are found in many countries. Mats develop in the shallow parts of coastal lagoons, where large fluctuations of temperature and salinity may occur (Stal et al. 1996; Villbrandt and Stal 1996).

4.2.4.2 Hypersaline Microbial Mats

Hypersaline environments can be found in shallow and sheltered coastal lagoons and tidal channels with high rates of evaporation and low precipitation. In the Mediterranean, hypersaline lagoons may form when they have narrow connections to the open sea and exchange of water is limited because a tide is virtually absent. Alternatively they can be totally disconnected from the sea and are fed by sea water through a sand bar as is the case in Solar Lake on the Red Sea coast of Sinai, Egypt. When virtually all water in such coastal lagoon environments evaporates, a natural salt pond develops, forming structures known in the Sinai desert as Sabkhas. In those geographical regions were the combination of sun and wind result in sufficient evaporation, artificial salt ponds have been constructed. Other hypersaline environments are inland seas which can be found at many different locations on the globe (Oren 1988) and in shallow lagoons of many of these lakes cyanobacterial mats develop (e.g. Zavarzin et al. 1993).

Cyanobacterial mats develop under these hypersaline conditions thanks to the potential of certain cyanobacteria to accumulate compatible solutes such as betaine that allow osmoregulation up to high salinities (up to 25%). Depending on the salinity cyanobacteria with different osmoprotectants prevail. Cyanobacteria are not found under saturating salinities or when the salt composition differs strongly from that of seawater. In general, hypersaline environments are also strongly alkaline. Cyanobacteria tolerate high pH thanks to their capacity of taking up bicarbonate and accumulating inorganic carbon. As a result of the fixation of CO_2 cyanobacteria generate alkaline conditions themselves. The best studied hypersaline microbial mats are from the salt ponds in Guerrero Negro, Baja California Sur, Mexico and Solar Lake, Sinai, Egypt (D'Antoni D'Amelio et al. 1989; Des Marais et al. 1992). Salts and brines are discussed more fully by Oren in Chap. 15.

4.2.4.3 Hot Spring Mats of Cyanobacteria

Thermal hot springs are environments in which the high temperature in combination with H_2S or acidic conditions decreases biodiversity enormously. Cyanobacterial mats are most common in hot springs at near neutral or alkaline conditions and are described more fully in Chap. 3. Cyanobacteria are generally alkaliphilic organisms. Acidic hot springs are more likely to inhabit eukaryotic microalgae. Thermal springs that contain sulphide may limit the formation of mats since thermophilic cyanobacteria do not tolerate the combination of high temperature and high levels of sulphide (Castenholz 1976, 1977). At moderate concentrations of sulphide, mats of *Oscillatoria* spp. have been shown to lower the sulphide concentration by anoxygenic photosynthesis by the cyanobacteria (Cohen et al. 1986; Ward et al. 1989). Another strategy is found in the so-called inverted mats (Castenholz 1976). Here, mats of the anoxygenic phototroph *Chloroflexus* overlay the cyanobacterial mat. Anoxygenic photosynthesis scavenges the sulphide and protects the underlying mat of the oxygenic heterocystous cyanobacterium *Chlorogloeopsis* sp. (Jørgensen and Nelson 1988). The maximum temperature at which photosynthesis can take place is slightly above 70°C.

4.2.4.4 Terrestrial Cyanobacterial Mats

Terrestrial cyanobacterial mats can be found in a variety of different environments. De Winder et al. (1989a, b) described a cyanobacterial-algal crust in coastal dunes. Sand dunes have a poor capacity of retaining water and are therefore extremely dry environments that are characterized by a low biodiversity. Under certain conditions there develops a mat of *Crinalium epipsammum*, a unique band-shaped filamentous cyanobacterium (Fig. 4.9); its unusual cell envelope is exceptionally well-adapted to desiccation (De Winder et al. 1990). This species is important in the Netherlands in stabilizing and protecting dune sand from wind erosion. Once this organism has established a matrix the community is taken over by the green alga *Klebsormidium flaccidum*.

Desiccation and life under low water potential are also the controlling factors for the development of cyanobacterial mats

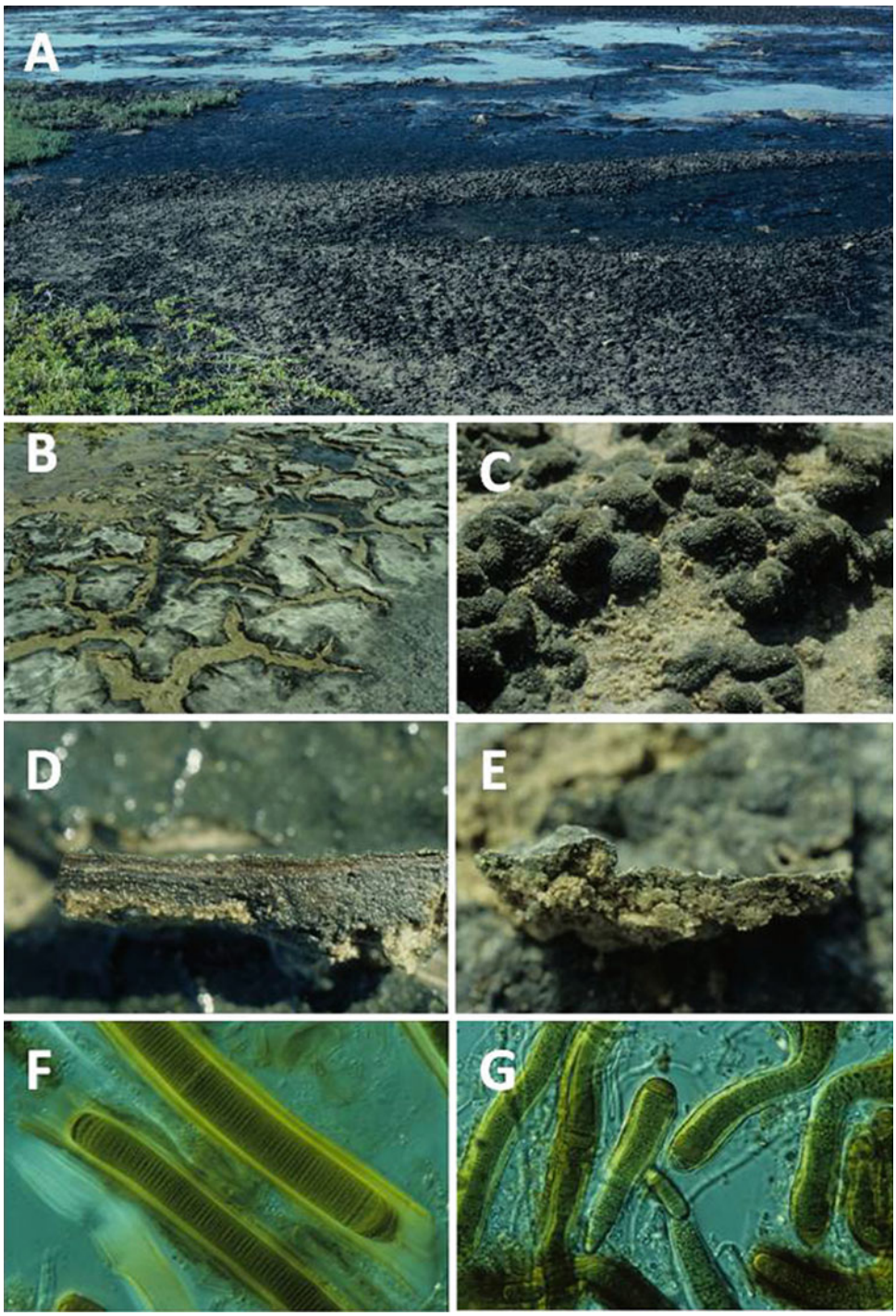

Fig. 4.7 Microbial mats on intertidal flats of the Pacific coast of Guerrero Negro, Baja California Mexico. (**a**) Two types of mat develop close to each other, with smooth mat (shown as *dark areas*) in the lower intertidal. (**b**) Smooth mat, showing cracks caused by desiccation at low tide. (**c**) Pustular mat. (**d**) Differences between the mats shown in cross-section: smooth mat has laminated structure typical of microbial mats – with a thin and dense layer of cyanobacteria on *top*, next a layer of anoxygenic purple sulphur bacteria, then a black layer of FeS, indicating that the mat is permanently anoxic below the layer of cyanobacteria. (**e**) Pustular mat showing a much looser mat of cyanobacteria on top, while layers of purple sulphur bacteria and FeS are absent, indicating that the sediment below the cyanobacteria is predominantly aerobic. (**f**) Smooth mat is composed of the non-heterocystous (but N_2-fixing) *Lyngbya aestuarii*, the trichomes of which are surrounded by a thick polysaccharide sheath and the organisms are embedded in a dense matrix of mucilage. (**g**) Pustular mat composed of *Calothrix*

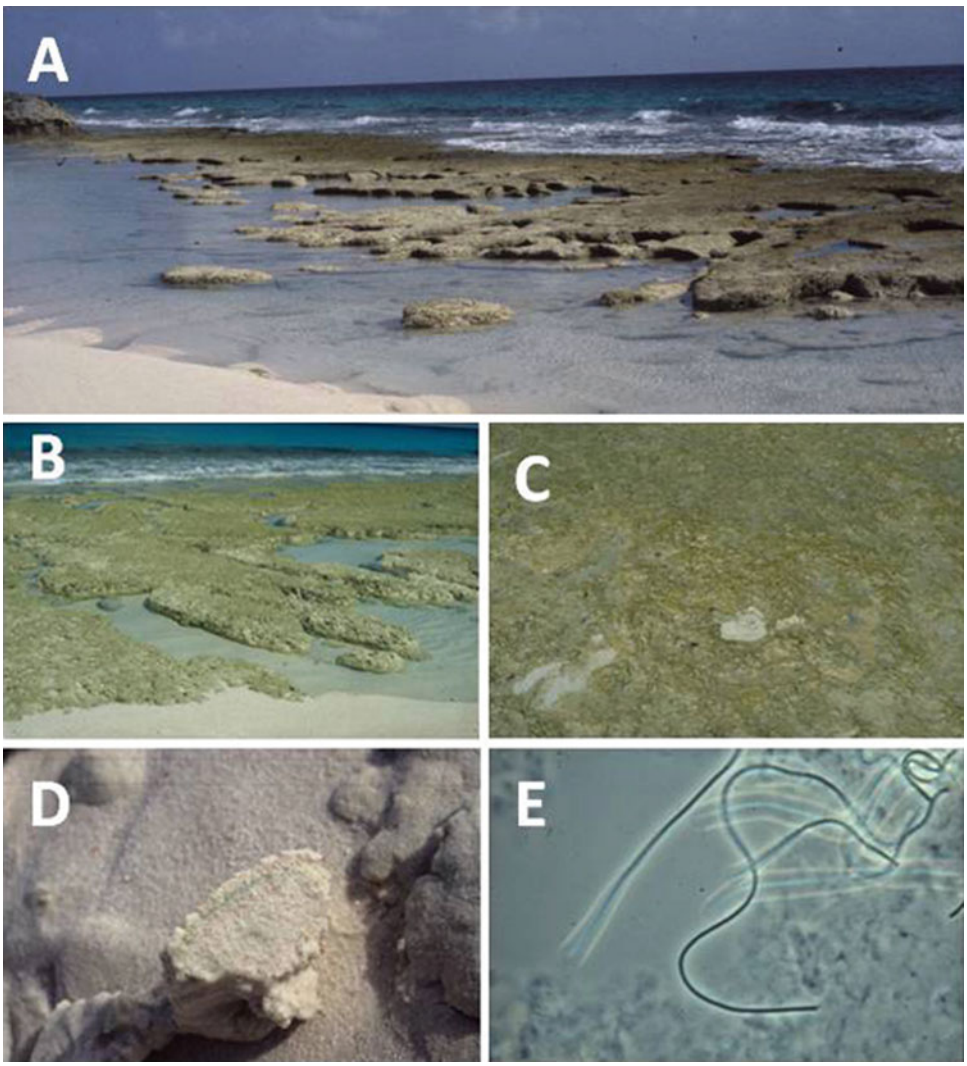

Fig. 4.8 (**a**) Stromatolites formed by cyanobacteria in the intertidal of Exuma Cays, Bahamas: these structures are considered as modern examples of known fossil stromatolites. (**b**) A closer look at these lithified sedimentary structures, which consist of trapped carbonate sediment cemented by micritic (microcrystalline) carbonate. (**c**) Surface of stromatolites is covered by a cyanobacterial-algal mat, which is thought to be involved in formation of the micritic horizons. (**d**) Cross-section of the *top* part of the stromatolite shows a distinct green layer of cyanobacteria. (**e**) *Schizothrix* is the dominant cyanobacterium in these modern stromatolites

and stromatolites in the hot desert. *Microcoleus chthonoplastes*, which occurs in some desert crusts (Brock 1975), has a polysaccharide sheath which plays an important role in protection from desiccation. After re-wetting the sheath absorbs water and the cyanobacterium resumes activity immediately (Campbell 1979). The sheaths of the unicellular desert *Chroococcus* sp. and *Chroococcidiopsis* sp. also have this function and these species are found hypolithically on rocks in the Negev desert (Potts and Friedman 1981; Potts et al. 1983; Caiola et al. 1993, 1996). Cyanobacterial mats are particularly well investigated in the Negev desert in Israel (Friedman et al. 1967; Berner and Jensen 1982). Krumbein and Giele (1979) found a calcifying mat of a unicellular cyanobacterium producing stromatolitic structures in the desert. Cyanobacterial mats also seem to be involved in the formation of rock varnish in the desert. Desert rock varnish is composed of iron and manganese oxides that are precipitated by the metabolic activity of mat microorganisms. The cyanobacterial mat is usually present underneath this hard brownish layer where they are protected from direct sunlight and are capable of retention of some water (Krumbein and Jens 1981).

Carbonate caves are other terrestrial environments that support the formation of microbial mats of the unicellular N_2-fixing *Gloeothece* (*Gloeocapsa*) and of the heterocystous *Nostoc* on walls that receive some daylight (or when artificial illumination is present) (Cox et al. 1981; Griffiths et al. 1987). Another example of such a low-light terrestrial environment is the mats of *Leptolyngbya* sp. described by Albertano and Kovacik (1996) on the walls of Casa Aureum

in Rome. Terrestrial mats of *Nostoc* have been reported from a variety of desert environments, including the cold desert in Antarctica (Davey 1983; Davey and Marchant 1983). Cyanobacterial mats from cold deserts have been described by Davey and Clarke (1992) and Vincent et al. (1993a, b).

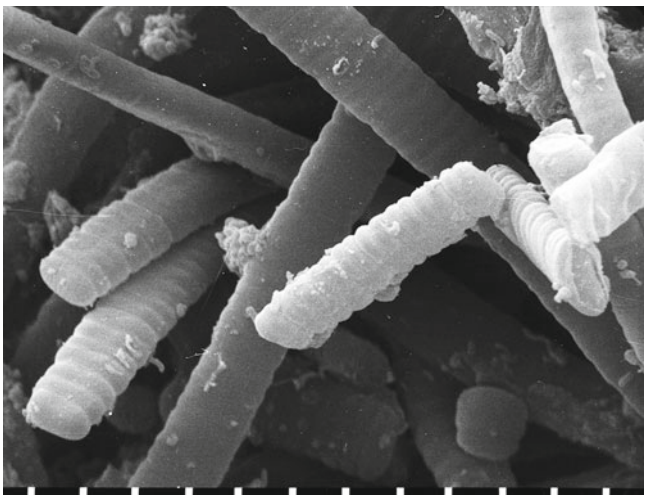

Fig. 4.9 Scanning electron micrograph of *Crinalium epipsammum*, a cyanobacterium forming phototrophic microbial crusts on coastal dunes. This organism is unusual because of the flat trichomes and the fact that the cell wall contains cellulose. Scale bar = 3 μm

4.3 The Organisms: Cyanobacteria That Build Microbial Mats

Cyanobacteria that build microbial mats include a variety of filamentous and unicellular species. The filamentous non-heterocystous *Microcoleus chthonoplastes* dominates marine intertidal microbial mats all over the world (Stal et al. 1985), hypersaline environments (Garcia-Pichel et al. 1996) and in the hot desert (Campbell 1979).

A notable characteristic of *M. chthonoplastes* is its occurrence in bundles containing many trichomes, often twisted like a rope. The bundles are enclosed in a common polysaccharide sheath (Fig. 4.10) which may be partitioned in different compartments (Fig. 4.10b). The rope morphology has been suggested to be an adaptation evolved to colonize unstable substrates (Garcia-Pichel and Wojciechowski 2009). Garcia-Pichel et al. (1996) investigated and compared cultures isolated from a variety of mats from geographically distant locations, both marine and hypersaline. Based on morphological and genetic characteristics, the authors concluded that all these isolates were closely related and belonged at least to the same genus and probably the same species.

The analysis of the 16S rRNA gene and morphological characteristics of a large number of strains that were assigned

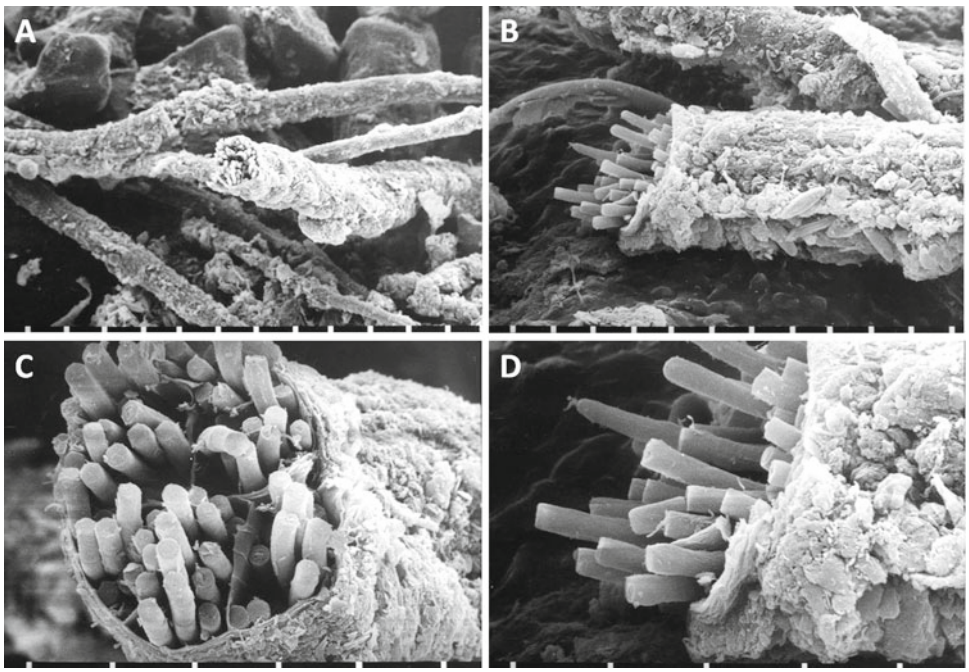

Fig. 4.10 Scanning electron micrographs of a mat of *Microcoleus chthonoplastes* of the island of Mellum, Germany. (**a**) Overview of the mat showing the large polysaccharide ensheathed bundles of trichomes. Scale bar = 30 μm. (**b**) Detail showing one end of a bundle of *M. chthonoplastes*. The *inner room* of the bundle is composed of different compartments separated by polysaccharide walls. This bundle contains a large number of trichomes. Scale bar = 10 μm. (**c**) A *side view* of a bundle of *M. chthonoplastes*. The *outside* is colonized by other microorganisms, including diatoms. Scale bar = 10 μm. (**d**) Detail of the end of the bundle with the individual trichomes sticking out. The trichomes can move freely in and out of the bundle. Scale bar = 10 μm

to *Microcoleus* led Siegesmund et al. (2008) to conclude that this genus belongs in fact to two families: the Oscillatoriaceae and the Phormidiaceae. The marine and hypersaline mat-forming *M. chthonoplastes* belongs to the latter and formed a coherent group that was proposed a new genus name, *Coleofasciculus* (only species so far *C. chthonoplastes*), in order to separate them from the freshwater and terrestrial *Microcoleus* (type strain *M. vaginatus*). However, in this Chap. 1 will refer to the better known name *M. chthonoplastes*.

More than 20 morphotypes of cyanobacteria were isolated from the mats of the intertidal sediments of the German North Sea island Mellum (Stal and Krumbein 1985) and a similar number with the same range of morphotypes were later isolated from a similar mat of the Dutch North Sea barrier island Schiermonnikoog. These also included several heterocystous species that were rarely observed in the field except in supratidal mats that received more freshwater than those in the intertidal regions. The cyanobacterial community composition of these mats varied considerably during the course of a year but also between different years. Sometimes the mats consisted virtually exclusively of *Spirulina* or *Merismopedia* (Palinska et al. 1996). Often these mats were composed of mixtures of different species (Fig. 4.11).

An important species that was always present and was frequently dominant was originally assigned to *Oscillatoria limosa* strain 23 (Stal and Krumbein 1985), which was shown to be the diazotrophic component of these mats. This strain was capable of aerobic N_2 fixation in culture (Stal and Krumbein 1981). Based on the 16S rRNA gene sequence analysis *O. limosa* was found to be related to *Lyngbya aestuarii*. This morphotype is observed frequently in microbial mats all over the world and as far as known all of these mats are diazotrophic. The trichomes have a thick polysaccharide sheath which is often pigmented (Fig. 4.7). Mats of *Lyngbya*/*Oscillatoria* can be found in geographically distant locations and are characterized by high rates of N_2 fixation.

Although many if not all microbial mats are capable of diazotrophy, the specialized heterocystous forms are only rarely the dominant component. That in some cases they can be isolated proves their presence, but obviously the prevailing conditions in the mat prevent their proliferation. Nevertheless, a few exceptions are known. In parts of the tidal flat in Guerrero Negro (Baja California Sur, Mexico) extensive mats of the heterocystous *Calothrix* sp. are present (Stal 1995) (Fig. 4.7). In a coastal lagoon near Bordeaux, France, mats of *Anabaena* sometimes develop (Villbrandt and Stal 1996). Mangroves often support extensive mats of the heterocystous *Scytonema* sp. (Potts 1979). *Calothrix* sp. is also known to form mats on rocks in the spray zone at the seashore (Whitton and Potts 1982). The development of these heterocystous diazotrophic mats is discussed further in Sect. 4.7.

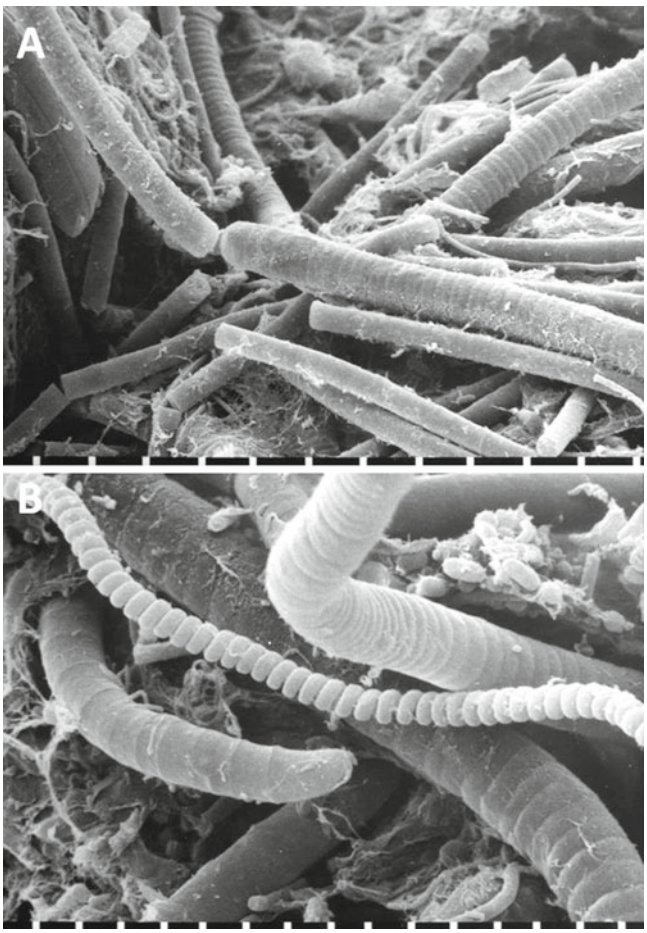

Fig. 4.11 (**a**) Scanning electron micrograph (SEM) of a N_2-fixing mat of *Oscillatoria* spp. and other cyanobacteria. (**b**) SEM photo of a detail of a mat of *Lyngbya* sp. with the typical coiled filament of *Spirulina subsalsa*, which is a typical component of these mats and a single trichome of *Microcoleus chthonoplastes*

Hot spring microbial mats such as Octopus Spring in the Lower Geyser Basin of Yellowstone National Park in the USA and similar microbial mats are dominated by the rod-shaped unicellular cyanobacterium *Synechococcus lividus* (Brock 1978). For a long time, strain *Synechococcus* sp. Y-7c-s was the only cultivated species. This strain was isolated from the 50–55°C Octopus Spring mat and considered to be representative for all thermophilic *Synechococcus* species since they possessed DNA with almost identical G+C ratios (Waterbury and Rippka 1989). In fact, at least seven different strains, all with identical morphology, are present (Ward et al. 1994). Y-7c-s was only detected in Clearwater Spring, which is slightly acidic (Ruff-Roberts et al. 1994; Ferris et al. 1996) and from which this strain may have been originally isolated. Hybridization probes have shown that *Synechoccoccus* sp. strain Y-7c-s was present in the Octopus Spring mats in extremely low frequency. Ferris et al. (1996) demonstrated that enrichment cultures selected for this strain.

Although morphological indistinguishable, the populations of *Synechococcus* sp. in Octopus hot spring microbial mats belong to a phylogenetic diverse group. Analysis of the 16S rRNA gene sequences of Octopus Spring revealed a high diversity of *Synechococcus* distantly related to *S. lividus*. These thermophilic *Synechococcus* are an ecologically diverse group of cyanobacteria that are distributed horizontally along a temperature gradient and vertically along light and oxygen gradients (Allewalt et al. 2006). However, by diluting the inocula prior to enrichment new strains of *Synechococcus* were obtained in axenic cultures. The different strains of hot-spring *Synechococcus* sp. have growth optima at different temperatures (Ward et al. 1994) and it was shown that their temperature ranges and optima were consistent with their distributions in the mats. Other adaptations may include those to pH and light.

In the hypersaline mats of Pond 5 of the Guerrero Negro saltern and the shallow flat mat of Solar Lake *M. chthonoplastes* is the dominant species and forms gelatinous organic mats (Fig. 4.5). Other cyanobacteria that may occur in these hypersaline mats are *Oscillatoria* sp. and *Spirulina* sp. Unicellular cyanobacteria may also be present. The Pond 5 mat of Guerrero Negro grows at salinities from 60‰ to 95‰. The salt content of the shallow flat mat of Solar Lake ranges from 45‰ to 180‰. At salinities that are permanently above 100‰ *M. chthonoplastes* does not proliferate well but *Spirulina subsalsa* may be found up to 150‰. At higher salinities up to 200‰, the unicellular *Aphanothece halophytica* usually dominates the mats (Dor and Paz 1989). The salinity tolerances for cyanobacteria seem to be higher in mats of the Sabkha, where *A. halophytica* occurs at 250‰, which is close to saturation, while *S. subsalsa* is present up to ~200‰ (Dor and Paz 1989). The reason for these differences is unclear. Salinity tolerance may be influenced by temperature. Although the sediment surface of the Sabkha may become hot from the solar radiation, the submersed mats in solar ponds may also be exposed to high temperatures. Therefore, halophilic cyanobacteria may also be moderately thermophilic such as was shown for a newly discovered organism with very thin trichomes of 1 μm *Halomicronema excentricum* that grows in the range of 3.2–12% salt and 28–50°C (Abed et al. 2002).

Lithified microbial mats found in the Exuma Cays, Bahamas, are built by the filamentous cyanobacterium *Schizothrix* (Pinckney et al. 1995). This forms thin trichomes about 1 μm wide, with cells 2–5 times as long as wide. The trichomes may be enclosed by a thick sheath. Communities of *Schizothrix* may form dense and tough mats that are often associated with calcium carbonate precipitation. The lithified microbial mats of Exuma Cays, Bahamas, result in the formation of stromatolites, a process which still goes on (Reid and Browne 1991; Pinckney et al. 1995; Reid et al. 2000).

4.4 Motility, Chemo- and Phototaxis of Cyanobacteria in Microbial Mats

Microbial mats are characterized by steep and fluctuating physicochemical gradients. In order to experience optimum conditions at all times, cyanobacteria must position themselves continuously in the mat. Microbial mats also often occur in environments with high sedimentation rates. This demands a light-oriented motility, in order to prevent permanent burial.

Cyanobacteria possess essentially three different ways in which they respond to light: phototaxis, photokinesis and photophobic response (Häder 1987a, b). Phototaxis is a movement, which is oriented along the direction of light. Phototaxis can be either positive or negative. Positive phototaxis is towards the direction of light whereas negative phototaxis is the movement away from the light. Both positive and negative phototaxis are important for cyanobacteria in microbial mats. Most cyanobacteria are adapted to growth at low light intensities. Excessive light may result in photooxidative stress and can cause damage. The combination of positive and negative phototaxis will allow the organism to obtain an optimum position in the mat. Most of the research on light responses of cyanobacteria has been carried out on *Phormidium* and *Anabaena* and more recently also on the unicellular *Synechocystis*. Little work has been carried out on light responses in cultures of mat-forming cyanobacteria.

Photokinesis is the term used for the phenomenon where speed of movement increases with light intensity. This is because of the greater supply of energy. Only positive photokinesis is known (negative photokinesis would be the decrease of speed at higher light intensities). The photophobic response is the reversal of the direction of movement as a result of a sudden change in light intensity. This response is very important for cyanobacteria. Both step-down and step-up responses are known (Häder 1987a). A step-down response causes the accumulation of the organisms in the light. At very high light intensities a step-up response may result in the accumulation of the organisms in a shaded area. Photophobic responses are clearly related to photosynthesis as could be concluded from action spectra (Häder 1988).

The light required for phototaxis might be extremely low (0.001 μmol photon m^{-2} s^{-1}) and also saturates at very low photon irradiance (1 μmol m^{-2} s^{-1}) (Ng et al. 2003). In most cases the action spectrum of phototaxis follows the photopigments of the cyanobacteria, the phycobiliproteins and chlorophyll *a* (Bhaya 2004). The low threshold for phototaxis is important for cyanobacteria in microbial mats to direct them to the surface after a large deposition event. The low light intensity and the complex action spectrum suggest that it is not required for providing the energy for locomotion,

which is confirmed by the ineffectivity of inhibitors of photosynthesis (Choi et al. 1999).

Motility is an extremely important property for most mat-forming cyanobacteria and occurs by gliding, which can be defined as a self-propulsion along a surface. This surface can also be the interior of the polysaccharide sheath. Trichomes may move forwards and backwards in their sheaths and may move out of it, leaving an empty sheath behind. Trichomes may also glide along each other. The hypotheses to explain gliding motility that have received most attention are:

(i) secretion of mucilage
(ii) contractile structures that cause surface undulations.

Some motile cyanobacteria possess junctional pore complexes, organelles that penetrate the cell wall and through which it is assumed that mucilage is secreted (Hoiczyk 2000). According to this hypothesis the mucilage adheres to the substrate and flows in tight contact with the trichome, thereby producing the propulsive force. The reversal of the movement would be obtained by using junctional pore complexes at the other end of the cell that are directed opposite. The rotation along the long axis in some Oscillatoriaceae could be produced through the presence of helically arranged fibrils. The arrangement of these fibrils determines indeed the left or right rotation, which is species specific. However, the highly motile *Phormidium uncinatum* does not possess junctional pore complexes, although it secretes polysaccharide (Häder 1987b).

Halfen and Castenholz (1971) and Castenholz (1973) suggested that the helically arranged microfibrils which they found in the external layers of the cyanobacterium *Oscillatoria princeps* can contract producing a surface wave that contacts the surface producing the force needed for the gliding movement.

Gliding movement is not restricted to filamentous cyanobacteria. The unicellular *Synechocystis* exhibits a motility that has been described as twitching and depends on type IV pili which moves the organism by pilus extension, adhesion and retraction (Bhaya 2004). Hence, gliding may well be a collection of different types of locomotion, each with their own specific mechanisms. One pelagic marine unicellular *Synechococcus* is capable of swimming, even if it lacks flagella (Waterbury et al. 1985). Swimming depends on swmA, a cell surface glycoprotein of *Synechococcus* and which is needed for the generation of thrust (McCarren et al. 2005). This mode of locomotion does not seem practical in the dense matrix of the microbial mat.

It is not certain how important the three different responses to light are in microbial mats. Ramsing and Prufert-Bebout (1994) concluded from light measurements in mats made by fiber-optic micro light sensors that light fields in microbial mats are uniform. This is caused by scattering of light, and it means that there is in fact no direction of light. Moreover, light intensity will not be subject to sudden changes in microbial mats. These authors therefore conceived that phototactic and photophobic responses would not be especially beneficial for mat-forming cyanobacteria. Studies with *M. chthonoplastes* indicated that the strategy of this organism is to minimize movement when conditions are favourable. Instead of varying the speed of movement (photokinesis) it moves less frequently. *M. chthonoplastes* also reverses its movement frequently. This is not a photophobic response because this would imply a step-down or step-up change in light intensity which is not the case. Ramsing and Prufert-Bebout (1994) further observed that *M. chthonoplastes* bends more frequently at optimum light intensity. In the long-term this could lead to curling of trichomes into bundles. Motility in such bundles is likely to be restricted. Such cyanobacteria are likely to be confined to a fixed position in the mat. In the hypersaline Guerrero Negro mats *M. chthonoplastes* was present throughout the mat and did apparently not migrate, whereas other bacteria, including other cyanobacteria, migrated through the mat and occupied different positions during the daily cycle (Dillon et al. 2009).

Garcia-Pichel et al. (1994) demonstrated that mat-forming cyanobacteria *Oscillatoria* sp. and *Spirulina subsalsa* migrated up and down in the mat in a daily manner (Fig. 4.12). At sunset these cyanobacteria moved towards the mat surface and stayed there throughout the night. At sunrise they migrated downwards. The depth to which they migrated appeared to be related to the light intensity, reaching the maximum depth in the mat at mid-day when the light intensity was highest. Interesting was also that *Oscillatoria* sp. and *S. subsalsa* contained unusually high amounts of chlorophyll *a* (3.9% d. wt). A unicellular cyanobacterium in this mat was non-motile and contained only 0.3% chlorophyll *a* (Garcia-Pichel et al. 1994). It was calculated that if *Oscillatoria* and *S. subsalsa* did not migrate they would be photoinhibited for most of the time, whereas the daily movement guaranteed optimum photosynthesis throughout the light period. Many cyanobacteria move deeper into the sediment at high light intensities (Pentecost 1984; Whale and Walsby 1984; Richardson and Castenholz 1987a) (Fig. 4.12).

Other researchers have also noticed that cyanobacteria migrate to the surface during the night or when the mat is shaded. Migration occurs also during the dark and Whale and Walsby (1984) therefore concluded that this upward movement was not controlled by light. Since these authors could not find any evidence for geotactic or magnetotactic responses, they assumed that migration of cyanobacteria was directed through chemotaxis in a chemical gradient. On the other hand, not all cyanobacteria are capable of moving in the dark. Malin and Walsby (1985) observed that motility of *Oscillatoria* sp. was strictly dependent on light and gliding stopped in the dark after a short period, presumably because energy reserves were exhausted. These authors demonstrated responses of *Oscillatoria* sp. to oxygen (aerotaxis) and CO_2 and bicarbonate. A light-dependent response to CO_2 would

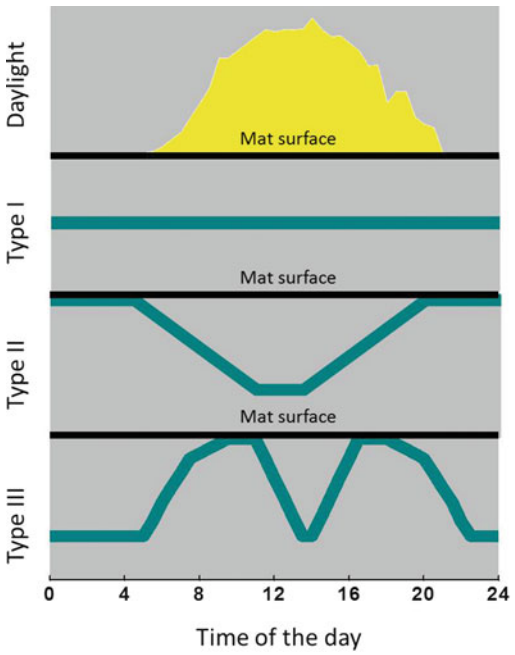

Fig. 4.12 Movements of cyanobacterial layer in mats during a 24-h period. *Upper panel* shows an example of the daily sinusoidal light curve. Example Type I is a mat which does not displace itself during a day-night cycle. This is either the case with unicellular cyanobacteria that are not motile and grow at optimal light intensity or by species that minimize movement when conditions are favorable, which may be the case in some populations of *Microcoleus chthonoplastes*. Example Type II is a mat that moves toward the surface at sunset and moves into the sediment during the day. *Upwards* movement may be controlled by chemical factors such as oxygen or sulphide. *Downwards* movement is in most cases attributed to negative phototaxis. Mats of *Oscillatoria* often show this type of displacement during a 24-h cycle. Example Type III is exhibited by the hot-spring *O. terebriformis*. In the dark the organism moves randomly, but motility is inhibited by sulphide, which eventually results in the accumulation of the population in the sulphide-rich layer deep in the sediment. Positive phototaxis occurs at low light and negative phototaxis at high light. This forces the organism to move deeper into the sediment during the middle of the day

be advantageous. Photosynthetic activity in microbial mats causes depletion of CO_2 and the high pH usually encountered in these environments as a result of photosynthetic activity and CO_2 fixation will shift the carbonate equilibrium resulting in even lower concentrations of CO_2. A light-dependent positive response to oxygen seems to be less advantageous. High concentrations of oxygen in the light may cause photo-oxidative reactions (Eloff et al. 1976) and photorespiration with therefore less efficient CO_2 fixation (Lorimer 1981; Reinhold et al. 1991).

Aerotaxis would be a useful strategy for dark migration. This would allow aerobic degradation of endogenous storage carbohydrate. The migration of *M. chthonoplastes* (Whale and Walsby 1984), *Oscillatoria* sp. and *S. subsalsa* (Garcia-Pichel et al. 1994) to the mat surface during the dark can be explained by a positive aerotaxis (Fig. 4.12). Alternatively, migration to the surface during the dark can be explained by a negative response to sulphide which is very toxic. In the dark the concentration of sulphide will increase because anoxygenic photosynthesis is absent and no oxygen is available for biological or chemical oxidation (Fig. 4.3). Castenholz (1982) therefore conceived a chemophobic response towards sulphide in cyanobacteria that migrate to the mat surface during the dark.

Chemotaxis in chemotrophic bacteria has received much attention but cyanobacteria have hardly been investigated for such migratory behaviour. Several cyanobacteria are capable of assimilating organic compounds such as glucose and fructose in the light (photoheterotrophy) and some even display a fully chemoorganotrophic metabolism (Smith 1982). *Oscillatoria terebriformis* is capable of fermenting extracellular compounds such as fructose and glucose (Richardson and Castenholz 1987b). Chemotactic responses of cyanobacteria to organic compounds are largely unknown. Fechner (1915) reported a negative chemotactic response to organic acids and Richardson and Castenholz (1989) observed inhibition of gliding of *O. terebriformis* by fructose. This effect was similar to that observed for sulphide. Glucose, the other substrate for this organism, did not have an effect, nor did lactate which is one of the fermentation products produced by *O. terebriformis*.

Cyanobacteria that form symbioses with plants were attracted by plant extracts, by certain sugars and particularly by mucilage (Nilsson et al. 2006). Higher temperature and darkness decreased chemotaxis, although this may be explained that light provides the energy for motility, rather than that it controls chemotaxis itself. Chemotaxis may be much more widespread in cyanobacteria than known until now, since operons for this process have been found in their genomes (Wuichet and Zhulin 2003) and the advantages for life in microbial mats with their steep and fluctuating chemical gradients are obvious.

An interesting behaviour has been encountered in *O. terebriformis*, which occurs in hot spring microbial mats and has a light-oriented motility. In the dark, this organism continues to move, albeit randomly. It may thus happen that it moves down into the sediment reaching the sulphide layer. Sulphide inhibits motility in *O. terebriformis* and therefore the organism is trapped in this layer (Richardson and Castenholz 1987a) (Fig. 4.12). Under laboratory conditions, 0.7 mM sulphide completely inhibited its gliding motility. Sulphide inhibited motility only in the dark or in the light when photosystem II was blocked by 3,-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). This inhibition was reversible and was abolished in the light. Since every individual organism has a high probability to become trapped in the sulphide layer, virtually the whole population will end up there. During the day the sulphide horizon will move down into the sediment relieving the inhibition of motility and at the same time the organism will move towards the light at the mat surface. At mid-day,

when light intensity is high, *O. terebriformis* shows negative phototaxis and moves deeper into the sediment in order to prevent photo-oxidative damage (Fig. 4.12). The majority of motile mat-forming cyanobacteria will prefer low light intensities and move deeper into the sediment during the day. The trapping of *O. terebriformis* in the sulphide layer during the dark is unusual, but essential for this organism to survive the dark period. In the presence of oxygen dark respiration will cause a rapid depletion of the endogenous storage carbohydrate which will result in the death of the organism in a matter of hours. The sulphide layer is of course anoxic. *O. terebriformis* is capable of fermentation and this process is slow, allowing for an extended period of energy generation. Indeed, the amount of energy that can be generated is small, but sufficient to cover its maintenance requirements (Stal and Moezelaar 1997). Most cyanobacteria, including mat-forming species, have low rates of dark respiration, allowing them to overcome long periods in the dark in the presence of oxygen. In the dark many microbial mats are virtually anoxic up to the surface, and fermentation is probably the only metabolism possible for the majority of cyanobacteria in the mat, with the exception of those that are exposed to the air. When the mat is submersed, oxygen decreases to zero within the diffusive boundary layer and no oxygen will be available to the mat (Fig. 4.3).

An unusual and new form of taxis is directed to gradients of water or water potential and has been termed hydrotaxis (Garcia-Pichel and Pringault 2001; Pringault and Garcia-Pichel 2004). Hydrotaxis has been found in a desert crust cyanobacterium related to a marine *Oscillatoria*. It was shown that migration depended on energy and probably occurred by gliding movement which is the mode of motility in this genus. When the surface of the crust dried out, the cyanobacteria migrated deeper into the crust where the water potential is higher. After a rain shower, the cyanobacteria moved quickly back to the surface. It is not known what exactly the cyanobacteria senses but it was speculated that it might be water potential or hydrophobicity (Pringault and Garcia-Pichel 2004). Migration towards water makes sense in microbial mats or crusts in desert environments but is probably unlikely in microbial mats in aquatic environments, even if coastal intertidal mats may become desiccated for prolonged periods as well. Hydrotaxis has hitherto only been described for this one occasion.

Mats of diatoms on intertidal mudflats in estuaries and bays have been reported to migrate into the sediments on a high tide (Serôdio et al. 1997). This migration might be under the control of an endogenous rhythm and was maintained for a certain period of time even when the trigger of the tidal cycle was taken away experimentally. For these diatoms it is important to migrate into the sediments when the tide comes in, in order to avoid grazing, even when this greatly limits their window for photosynthesis. In cyanobacterial mats such migration triggered by the tidal cycle has not been reported. Cyanobacterial mats are usually found in the higher reaches of the tidal flats and are not or for shorter periods inundated with water and therefore less subject to the tidal cycle. It is probably the lack of the tide-triggered migration that cyanobacteria can not escape grazing and therefore microbial mats do not occur there where diatom mats are found. In contrast to what had been assumed, it was demonstrated by Garcia-Pichel and Bebout (1996) that ultraviolet radiation penetrates well in microbial mats. The amount of penetration varies with the type of sediment on which microbial mats developed. Silty mud absorbed UV light most and quartz sand the least. Mats that are mainly organic take an intermediate position. UV light was absorbed in these mats more or less exponentially, in a similar way to visible light. There were two important aspects of the penetration of UV light in microbial mats, regardless of their sedimentological characteristics. In some mats the intensity of UV-B at the surface is considerably higher than the incident intensity; this is caused by scattering. Secondly, the total amount of UV-B in the euphotic zone of the mat ranged from 15% to 33% of incident irradiance which is high, particularly when compared with aquatic systems, where this number varies from 3% to 9%. These measurements carried out by Garcia-Pichel and Bebout (1996) were the first to demonstrate unequivocally that cyanobacterial mats develop under UV stress. Garcia-Pichel and Castenholz (1994) and Bebout and Garcia-Pichel (1995) provided also strong evidence that vertical migrations are partly under control of UV light. Garcia-Pichel and Castenholz (1994) reported that only 1.3 $W\,m^{-2}$ of UV-A (315–400 nm) was sufficient to keep the cyanobacteria *Oscillatoria* sp. and *Spirulina subsalsa* deep in the sediment. This intensity is only 3–4% of the level that these organisms would experience at mid-day. These cyanobacteria responded by negative phototaxis. In another study of microbial mats in Solar Lake (Sinai) it was shown that *M. chthonoplastes* responds clearly to UV-B light (310 nm). Exposure of the mat to 0.35–0.79 $W\,m^{-2}$ was sufficient to cause a downwards migration of the cyanobacteria. The effect of UV-B was about two orders of magnitude stronger than normal visible light. Also UV-A had this effect but was about five times less efficient than UV-B (Bebout and Garcia-Pichel 1995). It was concluded from these experiments that *M. chthonoplastes* is capable of sensing UV light, particularly UV-B.

There is no doubt that UV light causes serious damage to oxygenic phototrophic organisms (Cullen and Neale 1994) and has therefore negative effects on primary productivity (Smith et al. 1992). A mat-forming cyanobacterium will therefore benefit from the capability of sensing low levels of UV radiation and combining it with negative taxis. This will nevertheless result in a negative effect on total gross photosynthesis and productivity during exposure to UV light, but it is largely reversible (Bebout and Garcia-Pichel 1995).

Due to the downwards migration of the cyanobacterium, the biomass at the surface, and thus gross photosynthesis, decreases. In addition, surface photosynthesis may be partly inhibited by UV irradiation. Because in the deeper layers more biomass accumulates gross photosynthesis is even higher but due to the low level of photosynthetic active radiation (PAR), biomass specific photosynthesis is low. Not all cyanobacteria exhibit negative phototaxis with respect to UV light. Donkor and Häder (1991) and Donkor et al. (1993) showed that motility in the cyanobacteria they investigated was rather impaired by UV-B (280–315 nm). This may also have been the case in a mat of *M. chthonoplastes* of the temperate southern North Sea, where photosynthesis was strongly inhibited by UV-B radiation and did not recover during the subsequent 3 h when UV was excluded (Garcia-Pichel and Castenholz 1994).

Instead of migrating up- and down in a microbial mat, cyanobacteria have other possibilities to control the amount of light that they must absorb. Pierson and Parenteau (2000) observed for instance that cyanobacteria in the top layer oriented themselves vertically in the mat. This orientation may also have important consequences for the morphology of the microbial mat and may explain some of the morphologies of fossil stromatolites. *Merismopedia* is a unicellular cyanobacterium that occurs frequently in coastal microbial mats. It is characterized by its occurrence in plates in which the cells are well ordered. I have observed frequently that these plates may change its orientation from the large surface towards the light so that it receives maximum light to the side (one cell layer thick) to receive a minimum of light. The same was observed for the flat band-shaped filamentous cyanobacterium *Crinalium epipsammum* (de Winder et al. 1990).

4.5 Carbon Metabolism

4.5.1 Introduction

Cyanobacteria are the principal primary producers in the majority of microbial mats, although in some cases diatoms contribute as well. Oxygenic photosynthesis and sometimes anoxygenic photosynthesis and even chemosynthesis drives CO_2 fixation. Cyanobacteria enrich the microbial mat with organic matter. CO_2 fixation results in the formation of structural biomass of the cyanobacteria. This organic matter may become available to other organisms in the mat by the death and subsequent lysis of the cyanobacteria. However, it appears that, in spite of the high rates of photosynthesis usually observed, net growth of the cyanobacteria is often low in mature mats (Nold and Ward 1996). Hence, other processes must be involved in order to divert photosynthate to the mat community.

The benthic microbial mat community of the hypersaline lake, 'La Salada de Chiprana', northeastern Spain, produced dissolved organic carbon during the day and the night (Jonkers et al. 2003). These authors estimated that 14% and 49% of the mat gross and net photosynthetic production, respectively, diffused out of the mat in the form of low molecular weight fatty acids, although these compounds made up only 2% of the total dissolved organic carbon pool. The high flux of the dissolved organic carbon was generated by nutrient deficiency of the cyanobacteria. Photoheterotrophic *Chloroflexus*-like bacteria grew on top of the cyanobacterial mat at the expense of these phototrophic exudates. Also, large numbers of sulphate-reducing bacteria were found in the fully oxygenated surface layers. Another process that degrades dissolved organic carbon in microbial mats is by exposure of UV-B radiation (e.g. Häder et al. 1998). As will be discussed below, the flow of organic carbon from the cyanobacteria to the heterotrophic mat community may include the excretion of glycolate during photorespiration, the excretion of compatible solutes after an osmotic down shock, the excretion of fermentation products during dark anoxic conditions and the secretion of extracellular polymeric substances (EPS).

4.5.2 Oxygenic Photosynthesis

Oxygenic photosynthesis requires the presence of two photosystems (PS I and II). Cyanobacteria contain chlorophyll *a* in the reaction centers of both PS I and II, but the former contains about 2–3 times as many molecules of chlorophyll *a*. This chlorophyll may also contribute to the light harvesting, but the phycobiliproteins are far more important pigments as light-harvesting antennae. Jørgensen et al. (1987) demonstrated by recording photosynthetic action spectra in cyanobacterial mats that chlorophyll *a* contributed hardly to these action spectra even when additional 600 nm light was given to excite PS II.

Light is strongly attenuated in microbial mats, both by the sediment and by absorption by the dense phototrophic community. Sediments are transparent to light of long wavelengths (Stal et al. 1985). Dry sediments consisting of fine sandy quartz attenuate light much stronger than the same sediment saturated with water (Stal et al. 1985). Through the upper 1 mm of the latter more than 10% of surface irradiance penetrated, while this was only 2.5% of the dry sediment. The photic depth of the bare wet fine sandy sediment was about 4 mm. Through a 1.5 mm mat of cyanobacteria (0.5 g chlorophyll *a* m^{-2}), 0.45% of photosynthetically active radiation (PAR) penetrated. However, due to the specific absorption of the mat, wavelengths that would support oxygenic photosynthesis are specifically attenuated and oxygenic photosynthesis would not be possible below the cyanobacterial mat (Stal et al. 1985; Jørgensen et al. 1987; Pierson et al. 1987, 1990; Jørgensen and Des Marais 1988) (Fig. 4.4). Sediment and cyanobacterial mats are relatively transparent to light of

wavelengths above 700 nm, which explains the occurrence of communities of bacteriochlorophyll *a*-containing anoxygenic phototrophic purple sulphur bacteria (Pierson et al. 1987, 1990). These findings were confirmed by using fiber-optic microprobes connected to diode array detector (Kühl and Jørgensen 1992) and by a scalar irradiance microsensor which allowed spectral light measurements in sediments at the scale of the phototrophic microorganisms (Lassen et al. 1992a). More than 50% of the incident irradiance of 800 nm light penetrated a 1-mm thick microbial mat (Ploug et al. 1993). Lassen et al. (1992b) used this technique to study photosynthesis and photosynthetic efficiency in a microbial mat in Limfjorden, Denmark. This mat consisted of a top layer of diatoms and a cyanobacterial layer (*Oscillatoria* spp.) underneath. Using an oxygen micro-sensor, two peaks of oxygenic photosynthesis were found, corresponding to the diatom biofilm and the second deeper maximum corresponded with the layer of cyanobacteria. This latter maximum at 1 mm depth occurred at a light intensity of only 12 µmol photon m^{-2} s^{-1} i.e. 1.5% of incident light intensity. However, photosynthetic efficiency (rate of photosynthesis at a specific depth divided by the scalar irradiance at that depth) appeared to be tenfold higher in the cyanobacterial mat compared to the diatom film. This increased photosynthetic efficiency at low light intensity was the result of both the content of cyanobacteria at the depth of the second maximum of photosynthesis as well as a likely increased efficiency with which the available light was absorbed by the organisms (Lassen et al. 1992b). The report (Chen et al. 2010) that a cyanobacterium from a Shark Bay stromatolite can form a newly discovered chlorophyll, chl *f,* with the ability to absorb light in the infrared as well as red part of the spectrum, suggests the possibility that this pigment may also contribute to efficient use of light deeper in the stromatolite.

The dense biomass of cyanobacteria in the upper photic zone of microbial mats results in high rates of photosynthesis, and on a surface basis it compares to the productivity of rain forests, which are usually considered as the most productive ecosystems on Earth (Guerrero and Mas 1989) (Table 4.1). Revsbech et al. (1983) measured a total daily photosynthesis in a cyanobacterial mat in Solar Lake (Sinai) of 156 mmol O_2 m^{-2} and similar rates were found by Villbrandt et al. (1990) for a cyanobacterial mat in a temperate region (North Sea). The daily rates of photosynthesis measured by Revsbech et al. (1983) in Solar Lake microbial mats followed the light intensity during the day. The efficiency of photosynthesis was highest at low light intensity (up to 120 µmol photon m^{-2} s^{-1}). Photosynthesis was not inhibited at high light intensity. The same was found by Villbrandt (1992) when diurnal photosynthesis data on several days were plotted against light intensity (Fig. 4.13a). Photosynthesis increased in a linear way with light intensity. This photosynthesis versus light intensity curve of a microbial mat is completely different from isolated cultures of cyanobacteria (Fig. 4.13b). At low light intensity the photosynthesis curve is steep, depending on photosynthetic efficiency of the organism. At certain, usually moderate, light intensity photosynthesis saturates (P_{max}) and decreases again at higher intensity as a result of photoinhibition. The different *P* versus *I* curve of a mat is explained by the fact that all light is absorbed and used for photosynthesis. At higher light intensity cyanobacteria in the deeper parts of the mat will exhibit higher rates of photosynthesis. The diurnal variation of photosynthesis on a bright, cloudless day in July in a mature mat of *M. chthonoplastes* on the island of Mellum, North Sea (chlorophyll *a* content typically 0.3 g m^{-2}: Stal et al. 1985), showed a different pattern as the one measured by Revsbech et al. (1983). The rates of photosynthesis were highest during the morning hours and showed a sharp drop after mid-day (Villbrandt et al. 1990) (Fig. 4.14b). This mid-day drop in photosynthesis has been observed by other workers both in microphytobenthos as well as in phytoplankton (Paerl et al. 1989; Storch et al. 1990). An explanation for this observation may be that in the morning hours the concentration of dissolved inorganic carbon in the pore water of the mat is high as a result of decomposition of organic matter during the preceding night. After some hours of photosynthetic CO_2 fixation the pore water becomes depleted of dissolved inorganic carbon, which could explain the much lower rates of photosynthesis measured in the afternoon. A similar pattern as the one measured by Revsbech et al. (1983) was measured at another site of tidal flat on the island of Mellum. This site was characterized by freshly colonized sediment with *Oscillatoria limosa* (*Lyngbya aestuarii*) as the dominant species and with only 1/10 of the biomass compared to the mature mat (chlorophyll content typically 0.03 g m^{-2}, Stal et al. 1985) (Villbrandt 1992) (Fig. 4.14). Photosynthesis on a surface basis was much lower which is of course due to the much lower biomass and therefore the supply of dissolved inorganic carbon may have been sufficient throughout

Table 4.1 Comparison of primary productivity in microbial mats with other ecosystems (After Stal 1993)

Ecosystem	Primary productivity (mg C m^{-2} d^{-1})
Microbial mat	
Mellum (North Sea)	6,200
Solar Lake (Sinai)	5,000
Sea and ocean	
Mediterranean	60–500
Coastal upwellings	1,000–4,000
Ocean	<100
Lakes	
Oligotrophic lakes	40–80
Eutrophic lakes	300–3,000
Hypertrophic lakes	2,000–5,000
Mangrove forests	5,600
Rain forest	6,000

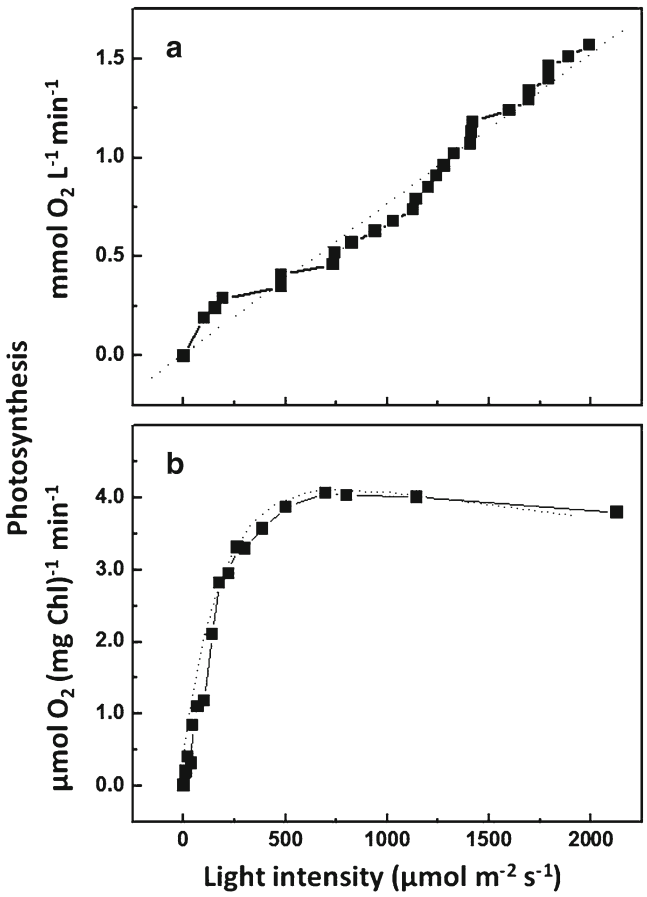

Fig. 4.13 (**a**) Photosynthesis versus light curve in a microbial mat of the North Sea island of Mellum (Germany) (Data from Villbrandt 1992). A large number of depth integrated measurements of photosynthesis recorded at different days and during different times of the day at the same location in the mat were used to plot in this *curve*. The curve was fitted by linear regression: P (mmol O_2 l^{-1} min^{-1}) = 0.01062 + (7.58 10^{-4}) I (R = 0.99 ± 0.08; N = 27 P < 0.0001). (**b**) Typical photosynthesis versus light intensity curve of a cyanobacterial culture

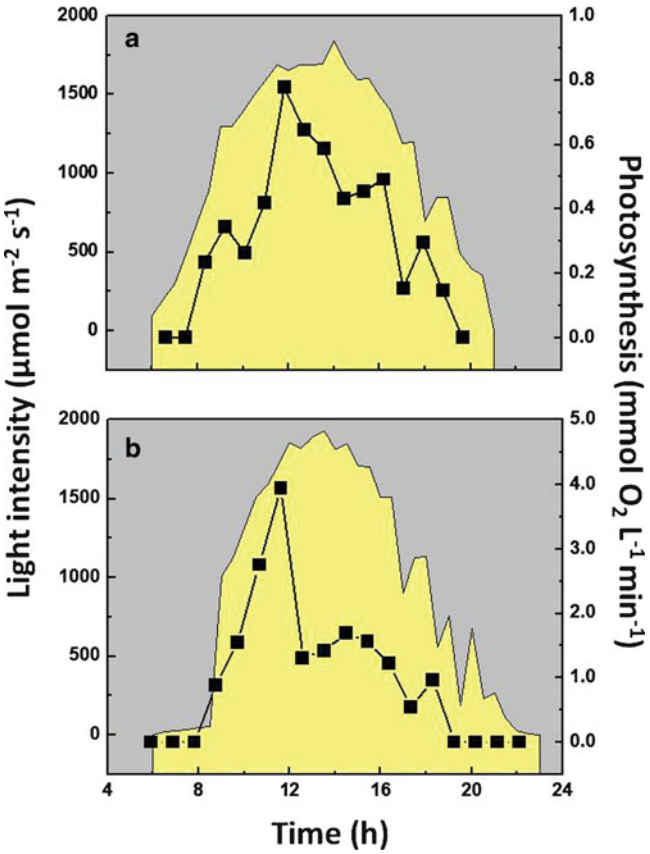

Fig. 4.14 Daily light curve (*shaded area*) and depth integrated photosynthesis of: (**a**) freshly colonized sediment with *Lyngbya* sp. as the dominant cyanobacterium; (**b**) mature mat of *Microcoleus chthonoplastes*. Both mats were located on tidal sand flats on the North Sea island of Mellum, Germany (Data from Villbrandt et al. 1990)

the day. Photosynthesis on a biomass basis was about twice as high as in the mature mat with comparable surface incident light intensity. This is attributed to the much smaller attenuation of light when biomass is low, i.e. the individual cyanobacteria receive much more light in the freshly colonized sediment compared to the mature mat.

The high rates of photosynthesis often observed in microbial mats may cause supersaturated oxygen conditions. The dense organic matrices represent a diffusion barrier that limits gas exchange. Oxygen bubbles that eventually develop may also be trapped in this matrix, causing the mats to become buoyant and lift off the sediments. Erosion of microbial mats as a result of this phenomenon can be regularly observed. Pieces of mat may be carried to vegetated areas and become desiccated when the tide has gone. Such desiccated pieces may be transported by wind over long distances. This phenomenon was first reported in 1686, becoming known as "Meteorpapier" because of the belief that it came from space. In his publication "Über das im Jahre 1686 in Curland vom Himmel gefallene Meteorpapier und über dessen Zusammensetzung aus Conferven und Infusorien" Ehrenberg (1838) identified this meteor paper as desiccated microbial mats.

4.5.3 Anoxygenic Photosynthesis

Anoxygenic photosynthesis in microbial mats is not the exclusive trait of purple- or green sulphur bacteria. Some species of cyanobacteria are capable of anoxygenic photosynthesis in which only photosystem I is involved. As with phototrophic sulphur bacteria, anoxygenic photosynthesis in cyanobacteria depends on sulphide as the electron donor. Roughly two categories of cyanobacteria can be distinguished with respect to the capacity of anoxygenic photosynthesis. In one group oxygenic photosynthesis is inhibited at low concentrations of sulphide and anoxygenic photosynthesis is induced. Inhibition of oxygenic photosynthesis by sulphide is

probably at the level of the manganese-containing, water-splitting enzyme (Oren et al. 1977). Both types of photosynthesis are mutually exclusive in these organisms. In the other group anoxygenic and oxygenic photosynthesis occur concurrently. At low sulphide concentrations oxygenic photosynthesis is more important and with increasing sulphide concentrations anoxygenic photosynthesis gradually takes over.

In both types of cyanobacteria, anoxygenic photosynthesis must be induced, a process which depends on a certain threshold of sulphide concentration and on light. Induction of anoxygenic photosynthesis in some organisms may take several hours. Therefore, cyanobacteria that possess the capability of carrying out oxygenic and anoxygenic photosynthesis concurrently have an ecological advantage in environments in which the sulphide concentration fluctuates, as is the case for instance in many marine and hypersaline microbial mats. Cyanobacteria that can carry out only one type of photosynthesis at a time are typical of environments with a constant supply of sulphide, as in certain hot spring microbial mats with an indigenous supply of sulphide. Moreover, these cyanobacteria tolerate higher concentrations of sulphide.

In cyanobacteria, anoxygenic photosynthesis is defined as energy generation through cyclic electron flow driven by photons absorbed by the reaction center of photosystem I and the fixation of CO_2 using sulphide as the electron donor. This means that this process also takes place when photosystem II is inhibited by the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) or when illuminated by far red light (>700 nm) which cannot be used by photosystem II. Cyanobacteria that perform anoxygenic photosynthesis oxidize sulphide to elemental sulphur according to the following stoichiometric equation:

$$12H_2S + 6\,CO_2 \rightarrow C_6H_{12}O_6 + 12S^o + 6H_2O$$

The elemental sulphur that is produced is deposited outside the cell but is often found attached to the outer sheath of the cyanobacterium as finely dispersed particles. The elemental sulphur is not further oxidized, as is the case in purple sulphur bacteria. In the mat-forming *Microcoleus chthonoplastes* (this particular strain is now assigned to *Geitlerinema* and is therefore closely related to the anoxygenic Solar Lake cyanobacterium '*Oscillatoria limnetica*', now also reassigned to *Geitlerinema*) thiosulphate was found to be the product of sulphide oxidation (De Wit and Van Gemerden 1987):

$$6H_2S + 3H_2O + 6\,CO_2 \rightarrow C_6H_{12}O_6 + 3H_2S_2O_3$$

While the oxidation of sulphide to sulphur yields only two electrons, the oxidation to thiosulphate yields four electrons per sulphide oxidized. Thus, the oxidation of sulphide to thiosulphate in *M. chthonoplastes* (*Geitlerinema*) seems to be twice as efficient as in other cyanobacteria in which zero-valent sulphur is the product. Rabenstein et al. (1995) reported that sulphite was the intermediate in those cyanobacteria that oxidized sulphide to thiosulphate. It is possible that thiosulphate is formed in a chemical reaction of sulphite with sulphide.

It is not clear why most anoxygenic cyanobacteria oxidize sulphide only to elemental sulphur. Phototrophic sulphur bacteria usually oxidize sulphide to sulphate, which yields eight electrons. However, in these bacteria the oxidation to elemental sulphur is usually much faster as the further oxidation to sulphate. Depending on the species this elemental sulphur is accumulated intra- or extracellularly. It can be hypothesized that the rapid oxidation of sulphide to elemental sulphur would be advantageous since it assures a rapid removal of the toxic sulphide. Moreover, in anoxygenic phototrophic bacteria, no matter whether they store the elemental sulphur intra- or extracellularly, it is not available for other organisms (Van Gemerden 1987). In that way, when sulphide is depleted these organisms continue anoxygenic photosynthesis at the expense of stored sulphur without competition with other organisms. In cyanobacteria the rapid consumption of sulphide most likely serves for detoxification since they can immediately switch to oxygenic photosynthesis when it has been completely oxidized. In addition, cyanobacteria can also use elemental sulphur as electron acceptor in anaerobic dark metabolism. *O. amphigranulata* is capable of using elemental sulphur for assimilatory purposes (Castenholz and Utkilen 1984). In the unicellular *Anacystis nidulans* low rates of CO_2 fixation are supported by the oxidation of thiosulphate (Utkilen 1976; Peschek 1978).

M. chthonoplastes (*Geitlerinema*) belongs to the group that performs oxygenic and anoxygenic photosynthesis concurrently. In this organism the growth rate decreased exponentially with increasing concentrations of sulphide. At a concentration of 1 mM sulphide (pH 8.0) growth was completely inhibited. Oxygenic photosynthesis was gradually inhibited with increasing concentrations of sulphide. The relative contribution of anoxygenic photosynthesis to total photosynthesis was >95% at concentrations of sulphide exceeding 0.35 mM (pH 8.0). The inhibition of growth at 1 mM of sulphide is probably caused by the complete inhibition of oxygenic photosynthesis, rather than anoxygenic photosynthesis. It was shown that *M. chthonoplastes* (*Geitlerinema*) requires oxygen for growth. When oxygenic photosynthesis was inhibited by DCMU, *M. chthonoplastes* (*Geitlerinema*) was not capable of sulphide-dependent anoxygenic phototrophic growth unless some oxygen was present. Therefore, a small contribution of oxygenic photosynthesis may be necessary in order to provide the essential oxygen. Oxygen may be required for the oxidation of fatty acids. *Anacystis halophytica*, for instance, possesses an oxygen-dependent

desaturation mechanism (Padan and Cohen 1982). Several cyanobacteria contain polyunsaturated fatty acids (Kenyon et al. 1972) and Padan and Cohen (1982) suggested that such cyanobacteria may be incapable of anaerobic growth. *O. limnetica* (*Geitlerinema*) does not contain polyunsaturated fatty acids which may explain its capacity for anaerobic growth (Padan and Cohen 1982). *M. chthonoplastes* (*Geitlerinema*) SAG 3192 contains considerable amounts of linoleate (18:2), linolenate (18:3) and tetradecadienate (14:2), regardless whether the culture was grown in the presence or absence of sulphide (De Wit et al. 1988).

The affinity of *M. chthonoplastes* (*Geitlerinema*) for sulphide is extremely low. The K_m for sulphide has been calculated as 974 μM, approximately the concentration at which growth of *M. chthonoplastes* (*Geitlerinema*) ceased (De Wit and Van Gemerden 1987). These authors analyzed the data of Jørgensen et al. (1986), who investigated the transition of anoxygenic photosynthesis to oxygenic photosynthesis in a mat of *M. chthonoplastes*. A K_m of 710 μM was calculated for sulphide oxidation in this mat. This affinity is close the one calculated in culture by De Wit and Van Gemerden (1987). These affinities for sulphide are extremely low when compared to the value of 5 μM for an anoxygenic phototrophic purple sulphur bacterium as *Thiocapsa roseopersicina,* which is frequently present in microbial mats (De Wit and Van Gemerden 1988). The similarity of the K_m of sulphide oxidation estimated in a culture to that estimated in a natural microbial mat of *M. chthonoplastes* indicates that this organism was probably responsible for the sulphide oxidation in the microbial mat. This was also suggested by Jørgensen et al. (1986) although they concluded this from the fact that purple sulphur bacteria constituted only a minor fraction in that mat.

The results on anoxygenic photosynthesis in a culture of *M. chthonoplastes* (*Geitlerinema*) obtained by De Wit and van Gemerden confirmed those for a natural mat by Jørgensen et al. (1986). These authors found that oxygenic and anoxygenic photosynthesis occurred concurrently. However, at higher concentrations of sulphide oxygenic photosynthesis was insignificant. Oxygenic photosynthesis in this mat started when the sulphide concentration decreased to about 0.3 mM, which is in agreement with the results obtained by De Wit and van Gemerden. The experiments of Jørgensen and co-workers showed that oxygenic photosynthesis could occur even when the microbial mat was exposed to 5–6 mM of sulphide in the overlying water. An oxygen peak was sandwiched between layers of sulphide. This sandwiching of cyanobacteria in microbial mats is often observed. Apparently, *M. chthonoplastes* is capable of resisting high concentrations of sulphide and recovers oxygenic photosynthesis. It is also capable of oxidizing this high concentration of sulphide in the light but this does not mean that the organism is indeed growing or even fixing CO_2.

Taking together the extremely low affinity of *M. chthonoplastes* for sulphide, the low growth rate with anoxygenic photosynthesis and the fact that this organism cannot grow in the absence of oxygen, the major function seems to be the detoxification of sulphide.

4.5.4 CO_2 Fixation

Carbon dioxide is the most important source of carbon for cyanobacteria and it is therefore crucial for the functioning of microbial mats. Cyanobacteria use the energy and low potential reductant (NADPH) produced during photosynthesis to fix CO_2 through the reductive pentose phosphate pathway (Calvin-Benson-Bassham cycle). The same pathway in the opposite direction, the oxidative pentose pathway, is used for the oxidation of storage carbohydrate during the dark in combination with aerobic respiration (Smith 1982; Schmetterer 1994).

High rates of photosynthesis will deplete the sediment of CO_2 and raise the pH. The pH may even reach values of over 9.5 (Revsbech et al. 1983) (Fig. 4.3) and any dissolved inorganic carbon will be present as bicarbonate or carbonate. Cyanobacteria are capable of adapting to growth at extremely low concentrations of dissolved inorganic carbon. Both CO_2 and bicarbonate are taken up by cyanobacteria. However, CO_2 is the substrate for RubisCO, the key enzyme of the Calvin-Benson-Bassham cycle and responsible for the fixation of CO_2. As is the case in other autotrophic organisms, this enzyme has also a low affinity for CO_2 in cyanobacteria, which means that both a high concentration of CO_2 and of RubisCO are prerequisites for the efficient fixation of carbon dioxide. Cyanobacteria possess an inorganic carbon-concentrating mechanism (CCM) which may result in up to 1,000-fold accumulation of inorganic carbon in the cell. A tentative model of this CCM in cyanobacteria proposes that either bicarbonate or CO_2 is taken up but that the former is the predominant species of inorganic carbon in the cytoplasm (Kaplan et al. 1994). Bicarbonate enters the carboxysome, a cell inclusion in autotrophic bacteria also known as polyhedral bodies. Carboxysomes contain virtually all RubisCO in organisms that possess these inclusions. The importance of carboxysomes for the CCM is also shown by the observations of Turpin et al. (1984) and McKay et al. (1992) that the number of these inclusions increases during adaptation of cyanobacteria to low CO_2 concentrations.

The fixation of CO_2 in microbial mats can be investigated by measuring the $^{12}C/^{13}C$ carbon isotope ratio in the organic matter. RubisCO discriminates between carbon isotopes with a slight preference for the lighter isotope ^{12}C. This fractionation factor α equals 1.029 (Roeske and O'Leary 1984), which means that organic matter may become 29‰ depleted in the heavy isotope ^{13}C when its origin is from RubisCO

mediated CO_2 fixation. This isotopic discrimination is only achieved when the CO_2 concentration is sufficiently high. This is generally not the case in microbial mats. Moreover, the measured value may differ from expected one because other organisms that were responsible for RubisCO independent CO_2 fixation may have been present in the system. Also cyanobacteria may fix significant amounts of CO_2 via alternative pathways such as PEP carboxylase or carbamylphosphate. Furthermore, the dissolved inorganic carbon produced from the decomposition of organic matter may be recycled and give rise to different net isotope discrimination. At the low concentrations of CO_2 that usually occur in cyanobacterial mats active transport of HCO_3^- becomes important (Badger and Andrews 1982) which results in a much smaller isotope discrimination than in the case of CO_2 uptake (Des Marais and Canfield 1994). Microbial mats are usually not much depleted in ^{13}C ($\delta^{13}C_{mat}$ is not very negative) because the pool of dissolved inorganic carbon is small compared to the rate of CO_2 fixation. This minimizes the isotope discrimination. The most negative values of $\delta^{13}C_p$ (photosynthate) are expected when CO_2 does not become depleted from the medium and when exchange between the medium and the site of fixation is rapid.

Des Marais and Canfield (1994) investigated the carbon isotope discrimination in two microbial mats in Guerrero Negro, Baja California, Mexico. The $\delta^{13}C_{mat}$ in these mats was slightly negative (−70‰). In the mat of *Lyngbya aestuarii* this value corresponded with the fractionation factor 1.007. This low value was evidently attributed to the closed reservoir behaviour of the system. The dissolved inorganic carbon that was produced by the mat during the night possessed the same negative value and therefore no changes in the $\delta^{13}C_{mat}$ were expected in the *L. aestuari* mat. In the mat of *M. chthonoplastes* photosynthesis did not discriminate between the lighter and heavier isotopes. At present a conclusive explanation for the negative value of $\delta^{13}C_{mat}$ in the *M. chthonoplastes* mat is not available (Des Marais and Canfield 1994). Processes such as excretion, fermentation and respiration do not change isotopic discrimination. Diagenesis of organic matter does not alter its isotopic composition (Des Marais et al. 1992) and the $\delta^{13}C_{DIC}$ is similar as $\delta^{13}C_{mat}$ (Bauer et al. 1991). It is known that the 'pond 5' mats of *M. chthonoplastes* are more or less in 'steady state', i.e. most of the organic matter that is produced by photosynthesis is mineralized in the mat. It is likely that photosynthesis scavenges very efficiently the dissolved inorganic carbon that is produced in the mat, thereby limiting net isotope fractionation. In fossil Proterozoic stromatolites $\delta^{13}C$ is much more negative than in present day microbial mats and stromatolites. This may reflect the higher levels of dissolved inorganic carbon in the Precambrian compared to today's concentrations (Kempe and Kazmierczak 1990a) or a more negative $\delta^{13}C_{DIC}$.

4.5.5 Photorespiration and Glycolate Excretion

During daylight, the dense phototrophic biomass in the cyanobacterial mat depletes CO_2 and accumulates oxygen, which sometimes may reach high supersaturation. It is assumed that such conditions will support photorespiration. Besides carboxylation, RubisCO also possesses oxygenase activity and can oxidize ribulose-1,5-bisphosphate to one molecule of each 2-phosphoglycolate (2PG) and 3-phosphoglycerate (3PGA) instead of two molecules of the latter during the carboxylation reaction (Lorimer et al. 1973; Lorimer 1981; Miziorko and Lorimer 1983). In fact, RubisCO has a much better affinity for oxygen as substrate than for carbon dioxide (Pierce 1988) and it has been suggested that the original function of the enzyme was an oxygenase rather than a carboxylase (Tabita 1988). Warburg (1920) discovered that O_2 inhibited CO_2 fixation in algae. Schau et al. (1950) showed that glycolate was a product of CO_2 fixation and Warburg and Krippahl (1960) demonstrated that its synthesis could be stimulated by oxygen. Glycolate is produced from 2PG by phosphoglycolate phosphatase and is metabolized via the glycine-serine (C2) pathway (Renstrom-Kellner and Bergman 1990), resulting in the production of 3PGA, CO_2 and NH_3. This light-dependent oxygen uptake and CO_2 evolution is called photorespiration. Photorespiration may represent a loss of fixed carbon which may be as high as 15–50% of net photosynthesis (Artus et al. 1986; Gerbaud and Andre 1987). What function of photorespiration is so important to justify this loss of fixed C?

In plants, the C2 pathway that metabolizes the toxic intermediate of photorespiration 2PG is essential for photosynthesis. Mutations in the C2 pathway result in plants with a high CO_2-requiring phenotype (high CO_2 would minimize photorespiration). In cyanobacteria the oxygenase reaction has been considered as irrelevant because these organisms possess a carbon concentration mechanism (CCM) which would allow sufficient high CO_2 concentration at the site of RubisCO. Cyanobacteria were known to convert 2PG to glycolate. However, it has now become clear that 2PG metabolism is probably present in all cyanobacteria, even in those with the smallest genomes, such as the marine picocyanobacteria *Prochlorococcus* and *Synechococcus*. The model strain *Synechocystis* PCC 6803 possesses three routes to metabolize 2PG: the plant-type C2 route, the bacterial glycerate pathway and the conversion of glyoxylate via oxalate to formate and subsequently to CO_2 (Eisenhut et al. 2008). It was also shown that 2PG metabolism was obligatory and that it could not be compensated by the CCM of this organism. Eisenhut et al. (2008) postulated that 2PG metabolism evolved simultaneously with oxygenic photosynthesis in cyanobacteria to allow them to cope with the toxic products generated by photorespiration that could occur because of the oxygen that accumulated in the cell and in the microbial mats, while

the CCM evolved only recently (Raven et al. 2008). Hence, the C2 pathway in plants was probably inherited from the cyanobacteria as well (Eisenhut et al. 2008).

Although it seems reasonable to assume that the metabolism of 2PG serves the elimination of toxic intermediates produced by the oxygenase reaction of RubisCO, there may be other benefits from photorespiration. 3PGA is regenerated for the Calvin-Benson-Bassham cyclus and other metabolic intermediates may be produced (Husic et al. 1987). The CO_2 produced may be re-fixed and the NH_3 may be re-assimilated even if this at the expense of ATP in both cases. In microbial mats photorespiration may help to prevent photooxidative damage by the lowering of O_2.

It is questionable whether in microbial mats the CCM would be sufficient to prevent photorespiration, because of the depletion in CO_2 during the daytime. If all cyanobacteria are capable of 2PG metabolism as is the case in *Synechocystis* PCC 6803, then it seems unlikely that glycolate is excreted, and this compound would therefore not be important as a substrate in microbial mats.

Many microbial mats are characterized by oxygen supersaturation in the light and CO_2 depletion and by very high pH (sometimes above 10). They are also subject to high light intensities and are chronically nitrogen depleted. All these factors will force the cyanobacteria to maximum rates of photorespiration. Glycolate metabolism, and therefore photorespiration, is closely associated with nitrogen metabolism. Renstrom-Kellner and Bergman (1989) demonstrated that the excretion of glycolate by *Anabaena cylindrica* decreased drastically in the presence of a source of nitrogen such as NH_4Cl or glutamate. As was shown by these authors, N_2-fixing cyanobacteria could lose up to 60% of photosynthetic fixed CO_2 as glycolate. Heterocystous cyanobacteria can access nitrogen through N_2 fixation. However, most mat-forming cyanobacteria are non-heterocystous and probably grow under severe nitrogen limitation. This suggests that these organisms may even lose the greater part of net photosynthesis. Bateson and Ward (1988) showed the importance of glycolate as a substrate for the microbial community in microbial mats. Glycolate may be utilized by sulphate-reducing bacteria, even in the presence of oxygen (Fründ and Cohen 1992). Glycolate-oxidizing sulphate-reducing bacteria have indeed been isolated from marine sediments (Friedrich and Schink 1993, 1995), but these organisms were strictly anaerobic.

4.5.6 Organic Compatible Solutes

Cyanobacteria exposed to high salinity or drought, accumulate osmoprotectors and extrude sodium ions through the activation or adaptation processes. These include (1) the uptake or biosynthesis of compatible solutes, (2) the active extrusion of sodium ions through the enhancement of ATPase activity, (3) modifications of the membrane lipid composition and (4) increase the energetic capacity through cyclic electron transport through photosystem I and through respiration.

In marine and hypersaline environments, micro-organisms accumulate solutes in order to obtain a sufficient turgor pressure necessary to allow cell division and growth (Taiz 1984). The cytoplasmic membrane is permeable to water and an organism that is exposed to an elevated salt concentration in the surrounding medium would tend to lose water. In order to retain water inside itself the cell can either take up ions until an osmotic equilibrium with the environment is obtained or accumulate low molecular weight organic solutes. High concentrations of inorganic ions are not compatible with the metabolism of cyanobacteria and cause inhibition of enzyme activity (Warr et al. 1984).

Cyanobacteria can be subdivided into three groups with respect to the type of organic solute they accumulate in response to osmotic stress (Reed et al. 1986a). Halotolerant freshwater cyanobacteria accumulate disaccharides (either sucrose or trehalose). Marine cyanobacteria accumulate the heteroside glucosylglycerol (2-O-α-D-glucopyranosylglycerol) and the very halotolerant hypersaline cyanobacteria accumulate quaternary ammonium compounds (glycine betaine and in one case glutamate betaine) (Mackay et al. 1984). There is no clear link between the type of solute and the taxonomic group of cyanobacteria, although all strains of *Anabaena* that were screened accumulated sucrose in response to osmotic stress. A habitat relation is suggested among species of the unicellular *Synechococcus.* Of the 33 strains investigated, all originating from freshwater environments accumulated sucrose, those isolated from marine systems accumulated glucosylglycerol and those from hypersaline habitats without exception betaine (Reed et al. 1986a). Stal and Reed (1987) screened 25 strains of cyanobacteria isolated from a microbial mat in the North Sea and found glycosylglycerol as well as trehalose and sucrose as osmolytes, suggesting no habitat relationship with the type of solute. Glucosylglycerol was nevertheless typically the dominant osmolyte in this marine ecosystem. The two dominant cyanobacteria in this mat, *M. chthonoplastes* (*Geitlerinema*) and *O. limosa* (*Lyngbya*) accumulated glucosylglycerol and trehalose, respectively. This property has been used to estimate the respective biomass of both species in these microbial mats (Stal and Reed 1987). Karsten (1996) measured the compatible solutes of a variety of strains of *M. chthonoplastes* isolated from various geographic locations and found that they contained glucosylglycerol as well as trehalose. However, the latter prevailed under sub-optimal salinities, while it appeared that the glucosylglycerol served as the only osmolyte. Betaine seems not to be limited to cyanobacteria from hypersaline environments since it has been identified in marine picocyanobacteria *Synechococcus* (Lu et al. 2006).

Although cyanobacteria normally accumulate a single low-molecular weight organic compound in response to osmotic stress, many species may produce a secondary compound. The synthesis of disaccharide is much faster than glucosylglycerol. Within 8 h of an osmotic upshock the disaccharide pool has reached 90% of its maximum, while with glucosylglycerol this is only the case after 24–48 h (Reed and Stewart 1988). Therefore the synthesis of disaccharide as secondary osmolyte may help for a quicker response to salt stress. Thus cyanobacteria that thrive under relative constant salinities may prefer glucosylglycerol while those that are exposed to fluctuating salinities may be better off with trehalose for example. This difference could explain why the pioneer in microbial mats, *Oscillatoria* (*Lyngbya*) sp., contains trehalose while the typical organism in established microbial mats, *M. chthonoplastes* contains glucosylglycerol. For the same reason hypersaline species contain sucrose in addition to betaine. Since betaine is a nitrogen-containing compound, nitrogen deficiency may also lead to the accumulation of sucrose as secondary osmolyte (Trüper and Galinski 1989). It has been shown that only glycine betaine provided a significant protection of enzyme activity against Na^+ ions, suggesting that sugars and polyols protect by a different mechanism (Warr et al. 1988).

Osmotic down shock exerted on betaine-containing *Aphanothece halophytica* resulted in the release of this osmolyte into the environment (Reed and Stewart 1988). This may have important consequences for an ecosystem such as a microbial mat because it may allow chemotrophic bacteria that cannot synthesize betaine to take it up from the environment (Reed and Stewart 1988). Moreover, betaine may serve as substrate for sulphate-reducing bacteria and the product of its metabolism, trimethylamine (TMA) is known as a so-called non-competitive substrate for methanogenic bacteria (Heijthuijsen and Hansen 1989).

Osmotic downshock in *Rivularia atra* resulted in a corresponding decrease of the osmoticum trehalose but only 10% was recovered from the medium and the rest was apparently metabolized or converted to glycogen (Reed and Stewart 1983). The glucosylglycerol accumulating strain *Synechocystis* PCC6714 and the sucrose-containing *Synechococcus* PCC6311 released 50% of their carbohydrates and over 70% of their amino acids after experiencing hypo-osmotic shock (Reed et al. 1986b). However, in some other cyanobacteria there is no evidence for the release of low molecular weight compounds upon hypo-osmotic shock (Reed and Stewart 1988). The release by cyanobacteria of low molecular weight compounds into a microbial mat would have a great impact on the ecosystem. The cellular concentration of these osmolytes is considerable and at full seawater salinity it may amount to as much 270 mM. These carbohydrates are easy accessible substrates for chemotrophic bacteria in the mat. Except in the case of a hypo-osmotic shock, which may occur in exposed microbial mats after a rain shower for instance, osmotica will also be liberated after death and lysis of the organism.

Microbial mats have often been found to evolve dimethylsulphide (DMS), a sulphur-containing organic volatile compound. It is known that DMS can be produced from dimethylsulphoniopropionate (DMSP) by microbial activity or by chemical decomposition at high pH (Kiene and Visscher 1987). DMSP occurs in a number of algae where its most likely function is that it serves as osmoprotectant (Turner et al. 1988). Vogt et al. (1998) suggested that some cyanobacteria might perhaps contain minor amounts of DMSP and Visscher and Van Gemerden (1991) suggested that *M. chthonoplastes* (*Geitlerinema*) may produce DMSP as a secondary osmolyte and could be the source of DMS in microbial mats. However, Van Bergeijk and Stal (1996) found a correlation between the number of diatoms in these mats and the amount of DMS that evolved from it. Some benthic diatoms accumulate large amounts of DMSP as osmoticum. It is possible that cyanobacteria take up DMSP from the environment (Vila-Costa et al. 2006).

4.5.7 Fermentation

When in microbial mats photosynthesis ceases, they may rapidly, sometimes even within minutes, turn anoxic. Cyanobacteria are essentially aerobic organisms that during the dark normally have a respiratory metabolism in which the endogenous storage carbohydrate glycogen is degraded (Smith 1982). When oxygen is absent, aerobic respiration is evidently not an option. Many cyanobacteria die and lysis occurs within 2–3 h after transfer to dark anoxic conditions. However, mat-forming cyanobacteria survive dark anoxic conditions for much longer time, often for several days. A number of these cyanobacteria were investigated in more detail and it was discovered that they were capable of fermenting glycogen. Fermentation as a constitutive property would have a number of advantages. Primarily, it would greatly increase the reactivity of the organism. Microbial mats are generally environments in which steep gradients of light and oxygen occur and these factors fluctuate strongly. If oxygen disappears rapidly, fermentation can immediately provide energy for maintenance, allowing the organism to survive. The excretion of fermentation products by cyanobacteria is an important process in microbial mats because it supplies other microorganisms, notably the sulphate-reducing bacteria, with substrate.

There is a great diversity of fermentation pathways in cyanobacteria. In some cases the pathways have been elucidated by the demonstration of the enzymes involved. In *O. limosa* (*Lyngbya*) the presence of the key enzymes of the homoacetate pathway has been demonstrated as well (Heyer et al. 1989).

Table 4.2 Cyanobacteria capable of fermentation (After Stal and Moezelaar 1997)

Organism	Strain, origin	Fermentation pathway	Products[a]
Anabaena azollae AaL	Symbiont from *Azolla caroliniana*	Homoacetate	Acetate (lactate, CO_2, H_2)
Anabaena azollae AaN	Symbiont from *Azolla caroliniana*	Homoacetate	Acetate (lactate, CO_2, H_2)
Anabaena azollae AaS	Symbiont from *Azolla filiculoides*	Homoacetate	Acetate (lactate, CO_2, H_2)
Anabaena siamensis As1	Paddy field	Homoacetate	Acetate (CO_2, H_2)
Cyanothece	PCC 7822 (Inst. Pasteur)	Mixed acid	H_2, ethanol, lactate, formate, acetate
Microcoleus chthonoplastes	Microbial mat	Mixed acid	H_2, ethanol, lactate, formate, acetate
Microcystis aeruginosa	PCC 7806 (Inst. Pasteur)	Mixed acid	H_2, ethanol, acetate
Nostoc sp. Cc	Symbiont from *Cycas circinalis*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. Al2	Symbiont from *Anthoceros laevis*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. Ef1	Symbiont from *Encephalartos ferox*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. MAC	Symbiont from *Macrozamia lucida*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. Mm1	Symbiont from *Macrozamia moorei*	Homoacetate	Acetate (lactate, CO_2, H_2)
Nostoc sp. M1	Symbiont from *Macrozamia* sp.	Homoacetate	Acetate (CO_2, H_2)
Nostoc sp. Gm	Symbiont from *Gunnera manicata*	Homoacetate	Acetate (lactate)
Nostoc sp. T1	Paddy field	Homoacetate	Acetate (formate, CO_2, H_2)
Nostoc sp. Bali	Paddy field	Homoacetate	Acetate (CO_2, H_2)
Oscillatoria limnetica	Hypolimnion Solar Lake	Homolactate	Lactate
Oscillatoria limosa	Microbial mat	Heterolactate Homoacetate	Lactate, ethanol, acetate
Oscillatoria sp.	Microbial mat	Not known	Lactate, ethanol, acetate, formate
Oscillatoria terebriformis	Hot spring microbial mat	Homolactate?	?
Spirulina (*Arthrospira*) *platensis*	Not known	Mixed acid	H_2, ethanol, acetate, formate, lactate
Spirulina sp.	Not known	Not known	Lactate, acetate

[a]Compounds in brackets are produced in minor quantities (From Stal and Moezelaar 1997)
?: uncertain

In the majority of pathways the Embden-Meyerhof-Parnas pathway (glycolysis) was involved in fermentation. Only the heterolactate fermentation makes use of parts of the oxidative pentose phosphate pathway. The oxidative pentose phosphate pathway is used by cyanobacteria during aerobic dark respiration and it is essentially the reverse of the reductive pentose pathway, which serves CO_2 fixation in the light (Smith 1982). In all cyanobacteria capable of fermentation the capacity for fermentation appears to be constitutive (Stal and Moezelaar 1997).

Stal and Moezelaar (1997) reviewed fermentation in cyanobacteria. Table 4.2 lists cyanobacteria capable of fermentation. The phenomenon was first discovered in *O. limnetica* (*Geitlerinema*) (Oren and Shilo 1979), which occurs in the sulphide-rich hypolimnion of Solar Lake, Sinai, and is typically adapted to anaerobic growth. In the dark this organism ferments glycogen to lactate. Since no other fermentation product was found, it was assumed that the homolactic acid pathway was used in this organism. In the non-heterocystous diazotrophic mat-building *O. limosa* (*Lyngbya*), heterolactic acid fermentation was found (Heyer et al. 1989). This organism produced equimolar amounts of lactate and ethanol from glycogen. In addition, it is capable of homoacetic fermentation, for which its osmoprotectant trehalose was used as substrate. Trehalose was degraded to 5–6 acetate and some hydrogen and CO_2. The occurrence of homoacetate fermentation in cyanobacteria is remarkable, since it further only occurs in a group of specialized anaerobic bacteria, the acetogenic bacteria. Homoacetate fermentation is energetically efficient. The occurrence of homoacetate fermentation has been proposed in a number of other cyanobacteria (De Philippis et al. 1996). The degradation of the osmoprotectant in *O. limosa* (*Lyngbya*) was another unexpected phenomenon. Trehalose represents a large amount of energy, which may be important for the organism to use under a situation of severe starvation. The question of how the organism compensates for the loss of compatible solute has not been answered, but it has been suggested that this may be through a temporary accumulation of inorganic ions such as K^+ (Stal and Moezelaar 1997). Also, the mat building cyanobacterium *M. chthonoplastes* (*Geitlerinema*) has been shown to ferment part of its osmoprotectant (Moezelaar et al. 1996). *M. chthonoplastes* (*Geitlerinema*) accumulates the heteroside glucosyl glycerol which is only degraded in cultures that contain low amounts of glycogen. Unlike in *O. limosa* (*Lyngbya*), *M. chthonoplastes* (*Geitlerinema*) possesses just one fermentation pathway. Glycogen and the glucose part of glucosyl glycerol are fermented via a mixed acid fermentation, resulting in the formation of formate, acetate, ethanol, lactate, H_2 and some CO_2. The presence of the homoacetogenic pathway allows

O. limosa (*Lyngbya*) acetogenesis from CO_2 and H_2 (Stal, unpublished observations). Acetogenesis from CO_2 has been observed in anoxic sediment (Hoehler et al. 1999).

Hydrogen is often a product of fermentation in cyanobacteria. Hydrogenases in cyanobacteria have been extensively reviewed by Tamagnini et al. (2002, 2007). Cyanobacteria possess different hydrogenases. N_2-fixing cyanobacteria produce hydrogen as a by-product of nitrogenase. Because nitrogenase obligatory produces hydrogen during N_2 fixation, aerobic N_2-fixing cyanobacteria usually possess an uptake hydrogenase. This enzyme carries out an oxy-hydrogen reaction. The third type of hydrogenase in cyanobacteria is reversible hydrogenase. This enzyme is frequently found in obligate anaerobic bacteria. Depending on the conditions it catalyses either the uptake or the production of hydrogen at approximately equal rates. Although its function in cyanobacteria has been debated for some time, reversible hydrogenase plays an important role in fermentation (Stal and Moezelaar 1997). Hydrogen concentrations are kept low in microbial mats because sulphate reducing bacteria, acetogenic bacteria, methanogenic bacteria and anoxygenic phototrophic bacteria use it as energy source and/or as electron donor (Hoehler et al. 2002). The escape of reduced gases from microbial mats into the atmosphere may have contributed to the oxygenation of the oceans on early earth (Hoehler et al. 2001).

Elemental sulphur may serve as electron acceptor in cyanobacteria. Many cyanobacteria have been shown to be able to reduce elemental sulphur to sulphide. It has been suggested that this process in *O. limnetica* (*Geitlerinema*) might represent a form of anaerobic respiration (Oren and Shilo 1979). However, in other cyanobacteria the advantage of the reduction of sulphide is probably that it serves as electron sink, allowing the formation of more oxidized product (acetate) which results in a higher amount of substrate phosphorylation.

Stal and Moezelaar (1997) have discussed the bioenergetics of fermentation in a number of different cyanobacteria for which sufficient information is available. Although evidently the amount of energy that is generated during fermentation is low, calculations showed that it usually exceeded the minimum amount required for maintenance. This remaining energy could potentially drive metabolic processes. For instance, *O. limosa* (*Lyngbya*) is even capable of maintaining a considerable rate of N_2 fixation under anaerobic conditions in the dark (Stal and Heyer 1987).

Hot spring microbial mats consisting of *Synechococcus* switched on a variety of genes involved in fermentation when in the dark and anaerobic conditions although the fermentation products were not identified (Steunou et al. 2006). This fermentation supported the fixation of N_2 that occurred in the dark in these mats. Anderson et al. (1987) investigated the fate of representative fermentation products (acetate, propionate, butyrate, lactate, and ethanol) in hot spring cyanobacterial mats. Fermentation occurred mainly in the top 4 mm of the mat. In the light, filamentous bacteria resembling *Chloroflexus aurantiacus* photoassimilated the fermentation products. In the dark under anaerobic conditions, only lactate was oxidized and also the extended incubation under these conditions did not enhance the metabolism of acetate, propionate, or ethanol. Acetogenic bacteria converted butyrate into acetate. In mats occurring at temperatures ranging from 50°C to 70°C acetate and propionate accumulated under dark anaerobic conditions.

4.5.8 Extracellular Polymeric Substances (EPS)

Extracellular polymeric substances are important components of microbial mats. They are involved in the attachment of cyanobacteria to the substrate and are essential structuring molecules by producing a matrix in which the organisms are embedded (Decho 2000). This polymeric matrix fulfils a number of other important functions in microbial mats, which will be discussed below.

Cyanobacterial exopolysaccharides are complex molecules composed of 6 or more different monosaccharides out of a suite of at least 12 sugars (De Philippis and Vincenzini 1998). The variety of linkages that is possible gives a broad range of possible structures. Cyanobacteria produce polysaccharides which can be roughly categorized in three groups: (i) endogenous polysaccharides that serve as storage compounds; (ii) cell envelope polysaccharides; (iii) extracellular polysaccharides. The endogenous polysaccharides in cyanobacteria can be found in the so-called α-granules, which are composed of a branched glycogen-like polymer. This polymer consists of α(1–4) and α(1–6) linked glucose molecules. The cell envelope consists of the cell wall polysaccharides and the external layers (glycocalyx). The glycocalyx can be subdivided in (i) the well-structured polysaccharide sheath, (ii) a polysaccharide capsule which extends outside the sheath but is clearly associated with the organism and is less structured and (iii) mucilage polysaccharide. The latter is not or very loosely associated with the organism. In fact, the polysaccharides that form the glycocalyx should all be considered as extracellular polysaccharides or exopolysaccharides (Fig. 4.16). Exopolysaccharides that are released into the surrounding environment may be the colloidal suspended molecules originating from any of the glycocalyx components. The different fractions are often poorly defined and mostly based on the different extraction procedures. Relatively little is known about cyanobacterial exopolysaccharides and their biosynthetic pathways are complex and not well known (Pereira et al. 2009).

Microbial exopolymers, including those produced by cyanobacteria, are high molecular weight mucous secretions that often have a complex structure. The molecular weight is

often more than 100,000 Da. Although polysaccharides are quantitatively the most important part of these exopolymers, other components are present as well. Proteins make up a significant part of the exopolymers (Decho 1990). These polymers are also known as extracellular polymeric substances (EPS). The composition and structure of EPS vary widely among different microorganisms (Tago and Aida 1977; Bertocchi et al. 1990; Decho 1994; Stal 1994) and even one single strain may produce more than one type EPS simultaneously or at different stages of growth (Christensen et al. 1985). Most of the polysaccharides in EPS are heteropolysaccharides that are composed of a variety of different monosaccharides, arranged in repeating units. EPS often contain uronic acids such as D-glucuronic acid, D-galacturonic acid and D-mannuronic acid. These are important functional groups because they contain carboxyl groups that are responsible for interactions with other EPS molecules or the binding of metals. However, other types of EPS are composed of neutral sugars. Extracellular polymeric substances may be hydrophilic or hydrophobic. Many are hydrophilic and may contain over 95% water by weight (Decho 1994). Depending on the chemical composition and the functional groups present, the tertiary structure of EPS is determined. The tertiary structure of EPS determines whether it is a cohesive gel or in a colloidal form. An intermediate form could be described as nonconsolidated mucilage (Decho 1994). The tertiary structure of EPS not only depends on the chemical composition but also strongly on temperature. Microbial mats and intertidal mudflats during emersion are subject to large variations in temperature and this will thus affect the cohesiveness and rheological properties of the sediment.

A large number of functions have been ascribed to EPS (Decho 1990). These include adhesion and immobilisation of the organism, protection against desiccation, protection from grazing, protection from toxic substances, scavenging of trace metals, and (anti-) calcification. Some of these functions will be discussed below as far as they are relevant to microbial mats.

Organisms in microbial mats are often subject to desiccation. EPS may retain large amounts of water and form a gel that stabilizes the macromolecular components and the cell structure of the cyanobacteria and organisms that produce it may overcome long periods of drought by forming hydrogen bonds with proteins, membrane lipids and DNA, thereby replacing the water shell surrounding these cell constituents (Caiola et al. 1996; Potts 1994). Some cyanobacterial EPS may be hydrophobic due to the presence acetyl-groups, peptide moieties or desoxysugars which determine the emulsifying properties and the rheological properties (Neu 1992). Particularly, EPS containing uronic acids or hydrophobic proteins may be important for micro-organisms, including cyanobacteria and diatoms, enabling these to attach to surfaces (Robins et al. 1986). For benthic organisms, it is important to stay on surfaces when conditions are optimal for growth. Some cyanobacteria are capable of modifying EPS from hydrophobic to hydrophilic and they may thus detach from a surface when conditions become inappropriate (Bar-Or et al. 1985). Benthic communities of diatoms may attach to the surface of intertidal mudflats by the production of hydrated and hydrophilic exopolymers during periods of emersion. During immersion, these polymers go into solution releasing the diatoms into the water column (Talbot et al. 1990). Benthic cyanobacteria may secrete flocculants, exopolymers that produce flocs with detritus and other material in the overlying water. These flocs eventually sediment, thereby clearing the overlying water and hence improving the conditions for these benthic phototrophs (Bar-Or and Shilo 1987, 1988).

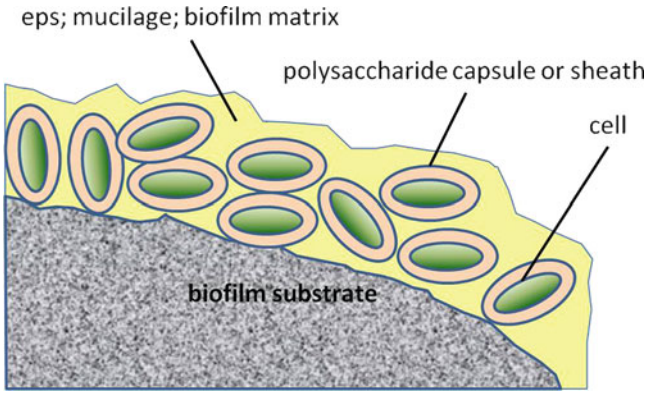

Fig. 4.15 Capsular and slime extrapolymeric substances (*EPS*) and the formation of a microbial mat

Mat-forming cyanobacteria that excrete EPS produce a matrix that stabilizes the sediment (Fig. 4.15). This is also the case with benthic films of diatoms that grow on intertidal mudflats (Paterson 1989; Stal 1994; Yallop et al. 1994). In the desert, hydrophobic EPS of microbial crusts cause the run-off of water preventing erosion (Mazor et al. 1996; Kidron et al. 1999).

Uronic acids are important components of EPS because these charged groups interact with sediment particles. Thus, EPS with a large content of uronic acids are more efficient in the stabilization of sediments (Martin 1971; Stal 1994). Sulphated groups and uronic acids contribute to the anionic nature of exopolysaccharides which determines the sticky properties of these molecules. EPS may also contain sulphated sugars. As the uronic acids, sulphate groups are also important for the tertiary structure of the polysaccharide and influence the stability of the microbial mat matrix (Decho 1990). Uronic acids as well as sulphate groups interact with a variety of metals. This may either result in the immobilization of toxic metals or scavenge trace metals that are important nutrients. The uronic acid groups of polysaccharides may be involved in the regulation of calcification. Sulphated polysaccharides are often encountered in algae but rarely in archaea and bacteria,

including cyanobacteria (Bertocchi et al. 1990). Nevertheless, sulphated polysaccharides have been found in cyanobacteria (Tease et al. 1991; Ortega-Calvo and Stal 1994) and a more thorough investigation of mat-forming cyanobacteria may reveal that such polysaccharides are more common in this group of organisms as previously thought (Pereira et al. 2009).

The polysaccharide produced by the mat-forming cyanobacteria fulfils an important function as a matrix for exoenzymes, plasmids and DNA (Decho 1990). Extracellular DNA is protected from DNases in the sediments (Romanowski et al. 1991), and may give rise to natural transformation in these ecosystems (Lorenz and Wackernagel 1990, 1994). Hence, in microbial mats, gene exchange may take place.

A considerable number of functions can be attributed to EPS but it is not clear what controls the formation of this polysaccharide and how this relates to one or more of the possible functions. It may very well be that the production of mucilage by cyanobacteria is the result of unbalanced growth caused by nutrient deficits (Lange 1976). Particularly a shortage or deficiency of nitrogen and sulphur results in the stagnation of protein synthesis while the full photosynthetic capacity remains. Under such conditions cyanobacteria accumulate large amounts of glycogen (Allen and Smith 1969; Lehmann and Wöber 1976). The capacity of the cell to store glycogen is limited and any additional polysaccharide may be excreted as mucilage. Old starved cultures often become viscous as a result of excess mucilage production. In the modern marine stromatolites of Highborne Cay, Bahamas, the maximum EPS production represented 7% of the total CO_2 fixation while most the fixed carbon was released as low-molecular weight dissolved organic carbon.

Little is known about the fate of EPS in mats. Some polysaccharides appear to be recalcitrant to microbiological degradation, whereas others are not. EPS that is newly formed in a microbial mat may be transformed rapidly (within 12 h) through the degradation by heterotrophic organisms, particularly by sulphate reducing bacteria (Decho et al. 2005). This is interesting because sulphate reducing bacteria are thought to degrade preferentially low-molecular weight organic compounds such as acetate, lactate and ethanol. The degradation of EPS was incomplete causing the accumulation of a more-refractory remnant polymer that was enriched in nitrogen. Net production of EPS in the Highborne Cay stromatolites was less than 2% of the total inorganic carbon uptake (Decho et al. 2005). A similar model of the origin of different fractions of EPS has been proposed for diatom biofilms (Stal 2010). In this model diatoms were supposed to produce one type of EPS which was enriched in glucose. Degradation and the preferential utilization of the glucose component left an EPS that was relatively depleted in glucose and enriched in uronic acids which was refractory and accumulated in the biofilm.

4.6 Calcification in Mats and Stromatolites

The biological control over calcium carbonate precipitation in the ocean leads to overproduction. It is estimated that 5 Gt calcium carbonate is annually produced in the ocean of which 3 Gt is removed from the system by incorporation and accumulation in sediments, while the other 2 Gt is dissolved (Milliman 1993). The weathering of rock on the continents causes a continuous runoff of calcium and carbonate into the sea. Therefore the oceans tend to be supersaturated with calcium carbonate. In order to maintain a steady state, the amount of calcium carbonate removed from the oceans must be the same as that entering. However, it is estimated that twice as much calcium is removed from the ocean by calcium carbonate precipitation than is brought in (Milliman 1993). This means that the ocean is not in equilibrium or that, sources and sinks are respectively under- or overestimated. The equilibrium of calcium carbonate in the oceans could be maintained by the dissolution of the excess calcium carbonate. Part of this dissolution is biologically controlled because it acts as a pH buffer for respiratory and fermentative processes. Another part dissolves in the deep sea, which is under saturated with calcium carbonate. Some calcium carbonate leaves the system by sinking as fecal pellets to the ocean floor or by fast burial. Although the surface waters of the ocean are supersaturated with calcium carbonate spontaneous precipitation does not normally occur.

Calcification is responsible for the lithification of microbial mats and is the basis of the formation of stromatolites. In most cases calcification seems under a stringent biological control, but the mechanisms by which living organisms influence the precipitation of calcium carbonate are poorly understood. Whereas the function of calcium carbonate precipitation in many organisms is obvious (e.g. shell or skeleton formation) this is not the case in microorganisms, including algae and cyanobacteria. Calcification in bloom-forming algae such as the coccolithophore *Emiliania huxleyi* would predominantly serve the production of CO_2 for subsequent photosynthetic fixation:

$$2HCO_3^- + Ca^{2+} \leftrightarrow CaCO_3 + CO_2 + H_2O$$

The so-called 'whitings', clouds of aragonite needles, that often occur in tropical lagoons and that have been considered as inorganic precipitates may be produced by the photosynthetic activity and CO_2 fixation of dense communities of picoplankton that increase carbonate ion (Robbins and Blackwelder 1992). Calcification in microbial mats may serve as a mechanism of producing CO_2 or it results from an increase in the concentration of the carbonate ion. Due to the dense phototrophic biomass and high rates of photosynthesis and the alkaline conditions it is likely that mats become

depleted in CO_2. Another function that has been proposed is the detoxification of intracellular calcium. But whatever the function, calcification can be generally inferred from the changes in the concentration of inorganic carbon and from the low solubility product of calcium carbonate.

Calcium carbonate is rather insoluble. Aragonite, which is often thought to be a product of biological calcium carbonate precipitation, has a solubility product (K_{sp}) of $10^{-6.19}$, and the more stable form calcite $10^{-6.37}$ (at 25°C and salinity of 35) (Zeebe and Wolf-Gladrow 2001). This means that when the ion activity product (IAP) (molar concentrations multiplied by their activity coefficients) of $\{Ca^{2+}\}$ and $\{CO_3^{2-}\}$ in a solution exceeds $10^{-6.19}$ calcium carbonate is saturated, although several-fold supersaturation is normally required for spontaneous precipitation (Arp et al. 2001).

$$\left\{Ca^{2+}\right\} + \left\{CO_3^{2-}\right\} \rightarrow CaCO_3$$

The concentration of calcium-ion in seawater is about 10^{-2} M (Ehrlich 1996). When the concentration of calcium ion is assumed to be constant, then the carbonate concentration determines calcification. CO_2 reacts with water to form bicarbonate and this dissociates according to the following reversible reactions:

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$$

Typical physicochemical processes that cause calcification are degassing of CO_2 and evaporation of water due to solar radiation. Evaporation leads to the formation of brines in which minerals precipitate, of which calcium carbonate is only a minor component (Yechieli and Wood 2002). In hot spring microbial mats degassing of CO_2 shifts the equilibrium towards the carbonate ion and to the formation of travertine deposits (Fouke et al. 2000).

In microbial mats, a number of biological processes influence the equilibriums of carboxy species and hence may control calcification (Dupraz et al. 2009). These include specific metabolic processes in which CO_2 is consumed such as photosynthesis, chemosynthesis and, less importantly, heterotrophic CO_2 fixation. Metabolisms in which CO_2 is produced such as respiration and fermentation may cause an acidification of the medium and eventually result in dissolution of calcium carbonate rather than precipitation. Nevertheless, Krumbein (1974) demonstrated the formation of aragonite on the surface of marine bacteria as the result of their metabolism of substrates such as glucose, sodium acetate and sodium lactate. However, this also strongly depends on the environmental conditions that apply (Canfield and Raiswell 1991). A variety of metabolic processes influence the equilibrium of inorganic carbon by the production of acids and bases.

Photosynthesis is an important process in the vast majority of microbial mats, at least in those occurring in illuminated environments. Because photosynthesis normally involves CO_2 fixation it has often been considered important for calcification (Golubić 1973; Krumbein and Giele 1979; Pentecost 1988). The fixation of CO_2 from a bicarbonate solution will result in an increase in carbonate ion:

$$2HCO_3^- \leftrightarrow CO_2 + H_2O + CO_3^{2-}$$

Other important metabolic processes in microbial mats which remove CO_2 are chemosynthesis and heterotrophic CO_2 fixation. Organisms that carry out chemosynthesis include colourless sulphide oxidizing bacteria, nitrifying bacteria, autotrophic sulphate-reducing bacteria as well as methanogenic- and acetogenic bacteria. Heterotrophic CO_2 fixation occurs in virtually all organisms but is limited and negligible compared to the CO_2 produced during the oxidation of organic compounds. This process is therefore not important for calcification. Many reports mention calcification as a result of photosynthesis by cyanobacteria (e.g. Golubić 1973; Krumbein and Giele 1979; Pentecost 1988; Pentecost and Bauld 1988). Calcification associated with anoxygenic photosynthesis or anoxygenic phototrophic bacteria has only been reported in one case in which the purple non-sulphur bacterium *Rhodopseudomonas palustris* was shown to stimulate calcification in a solution that was oversaturated with calcium carbonate (Bosak et al. 2007). Phototrophic sulphur bacteria produce sulphuric acid and they will therefore cause calcium carbonate dissolution rather than its precipitation although anaerobic sulphide oxidation has been reported to be involved in calcium carbonate precipitation in a marine stromatolite (Visscher et al. 1998). The same holds true for most chemosynthetic metabolisms and for heterotrophic CO_2 fixation. Precipitation of calcium carbonate may be indirectly associated with oxygenic photosynthesis and due to an increase of pH and/or a shift in the equilibrium of inorganic carbon (Golubić 1973; Krumbein and Cohen 1977). This was elegantly demonstrated in a hypersaline microbial mat (Ludwig et al. 2005). These authors showed that calcification was solely due to the increase of the carbonate ion as the result of photosynthesis and that the changes in the activity of calcium were not important. The contribution of heterotrophic bacteria was indirect as these organisms kept the concentration of dissolved inorganic carbon high in the pore water. Sulphate reducing bacteria did not change the pH and their effect was solely maintaining high concentrations of dissolved inorganic carbon. On the other hand, Chafetz and Buczynski (1992) found that calcification in stromatolithic microbial mats was associated with heterotrophic bacteria rather than

with the cyanobacteria and these seemingly contradictory observations emphasize the complexity of the process and the fact that the actual environmental conditions may cause quit different outcomes.

Aerobic or anaerobic oxidation of organic compounds results in the production of CO_2 and/or HCO_3^- and affects pH and consequently causes a shift in the equilibrium of inorganic carbon. Organic matter possessing the "Redfield" stoichiometry of C:N:P of 106:16:1 is oxidized by O_2, NO_3^- and SO_4^{2-} according to the following reactions (Boudreau and Canfield 1993):

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138O_2 \rightarrow 122H_2O + 106\,CO_2 + 16\,NO_3^- + 16H^+ + H_3PO_4$$

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 94.4NO_3^- \rightarrow 84.8H_2O + 13.6\,CO_2 + 92.4\,HCO_3^- + 55.2N_2 + H_3PO_4$$

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 53SO_4^{2-} \rightarrow 106\,HCO_3^- + 53H_2S + 16\,NH_3 + H_3PO_4$$

The formation of CO_2 and the acidification of the medium could result in the dissolution of calcium carbonate rather than cause its precipitation. Anaerobic respiration results in the formation of bicarbonate and could give rise to supersaturation of calcium carbonate. The effects of the sequential oxidation of organic matter by the three electron acceptors oxygen, nitrate and sulphate on pore water pH and calcium carbonate saturation are complex and depend on the prevailing conditions (Boudreau and Canfield 1993).

In the microbial mats of Solar Lake (Sinai), it has been shown that sulphate reduction and $CaCO_3$ formation were stoichiometrically related and organic carbon was transformed into a number of different carbonate minerals (Jørgensen and Cohen 1977; Krumbein and Cohen 1977; Krumbein et al. 1977). However, whether sulphate reduction in microbial mats in reality results in calcium carbonate precipitation depends largely on a variety of conditions that prevail in these microbial mats. Most important is the development of alkaline conditions, the removal of excess CO_2 or the presence of a suitable buffer (Ehrlich 1996). The precipitation of sulphide as iron sulphide acts as a pH buffer. In the absence of iron, sulphate reduction produces equal amounts of H^+ and HCO_3^-, which will thus cause a decrease of carbonate saturation. In many microbial mats high rates of sulphate reduction occur but despite this, calcification is absent. In the modern stromatolites of the Exuma Cays the tightly associated sulphate reduction and anaerobic sulphide oxidation promoted calcification, while the couple oxygenic photosynthesis and aerobic respiration cause calcium carbonate dissolution (Visscher et al. 1998).

Modern microbial mats are often considered as the structural analogues of Precambrian stromatolites. By definition stromatolites are lithified laminated formations. Precambrian stromatolites were formed in shallow marine areas. Lithification of present day coastal microbial mats is extremely rare and it is still an enigma why this should be so. Kempe and Kazmierczak (1990a, b) and Kazmierczak et al. (1996) investigated stromatolites in the sea-linked Satonda Crater Lake in Indonesia and alkaline Lake Van in Turkey, both formed under extreme alkaline conditions. They hypothesized that the greater abundance of stromatolites during the Precambrian should be attributed to the much greater alkalinity of the marine environment during that era (Kempe and Kazmierczak 1990a). The hypothesis of a Precambrian soda ocean may certainly offer an explanation for the greater abundance of stromatolites and the discovery of modern calcifying stromatolites in alkaline seas supports this. Nevertheless, calcification in these stromatolites is still under biological control rather than being a spontaneous occurrence. Moreover, other recent stromatolites are formed under less alkaline or normal marine conditions such as those found in the French Polynesian atolls (Défarge et al. 1994a, b) or in the Bahamas (Reid and Browne 1991). Even if the early oceans were more alkaline, the marine environment today is still supersaturated with calcium carbonate. Furthermore, in microbial mats several biological processes predominate which presumably increase the concentration of carbonate ion, which theoretically should lead to calcium carbonate precipitation. As a result of active photosynthesis and CO_2 fixation in the top layer of cyanobacterial mats the pH in these mats may reach values as high as 9.5 (Fig. 4.3) (Revsbech et al. 1983). Although these conditions would normally promote calcification, this does not happen in most marine microbial mats.

There is also abundant evidence of non-lithifying Proterozoic microbial mats that are recognized as microbially induced sediment structures (MISS), suggesting that the past may not have been so different from the present (Noffke et al. 2006; Noffke 2009). An experiment in which a non-lithifying mat was transplanted into an environment with lithifying microbial mats showed that it was now capable of calcification (Dupraz et al. 2009). Kremer et al. (2008) showed abundant calcification in an otherwise typically non-lithifying coastal microbial mat. This indicates that calcification is probably not so unusual and that the absence of lithification of these mats may be due to a subsequent dissolution of the calcium carbonate.

Hence, spontaneous calcification does not seem to be important in stromatolites. Biological control of calcification may not only exist in the change of the carbonate ion concentration and equilibrium but also in a mechanism that inhibits calcification (anti-calcification) (Westbroek et al. 1994). Biologically controlled calcification must distinguish

between supersaturation of calcium carbonate in a solution (which is the thermodynamic force) and those factors that influence the kinetics of the process. The latter may be either inhibitory or stimulatory factors. Supersaturation of calcium carbonate in the ocean is the primary driving force of calcification that can be dramatically increased in the immediate vicinity of phototrophic organisms. Because uncontrolled calcification in or around organisms is unwanted it may be clear that some mechanism must exist that can inhibit the process. Crystal poisons such as Mg^{2+}, SO_4^{2-}, PO_4^{3-} that complex with CO_3^{2-} and Ca^{2+}, respectively, are not sufficient and additional mechanisms must be postulated. It is known that some small acidic molecules may inhibit crystallization. An example is the binding of Ca^{2+} to oxalate (Verrecchia et al. 1990). Acidic polysaccharides are also very effective in binding Ca^{2+} or interact with it (Dupraz and Visscher 2005; Braissant et al. 2009). These interactions will doubtless influence calcification. Figure 4.16 depicts the way in which such polysaccharides could influence crystallization or crystal growth (Westbroek et al. 1994). The association of a polyanion with Ca^{2+} ions will lower the activity of the latter below the saturation of calcium carbonate and prevent subsequent crystallization. Likewise, polyanions may associate with a growing calcium carbonate crystal and prevent its further growth. A layer of charged polymers may bind calcium carbonate crystal and arrest its growth. The structure of such polymers may determine crystal shape. Evidence has been obtained that this mechanism is involved in the formation and morphology of coccoliths in the coccolithophore *Emiliania huxleyi* (Borman et al. 1982, 1987).

In microbial mats it is hypothesized that extracellular polymeric substances (EPS) which are mainly composed of polysaccharides serve as agents that inhibit calcification. EPS produced by cyanobacteria are often rich in uronic acids and contain other acidic groups such as pyruvate, succinate, sulphate and phosphate groups and hence are negatively charged polyanions (Bertocchi et al. 1990; De Philippis et al. 2001; Sutherland 2001). Many microbial mats are composed of vast amounts of EPS in which the cyanobacteria and other organisms are embedded. It is possible that this EPS acts as an anti-calcification agent. Cyanobacterial EPS may bind 55–183 mg Ca g^{-1} EPS (Li et al. 2001; Ortega-Morales et al. 2006). When heterotrophic bacteria decompose this EPS, high concentrations of calcium carbonate may exist locally, leading to precipitation (Dupraz and Visscher 2005). In some non-lithifying microbial mats such as in Solar Lake (Sinai), aragonite needles are formed in the deeper layers of the mat,

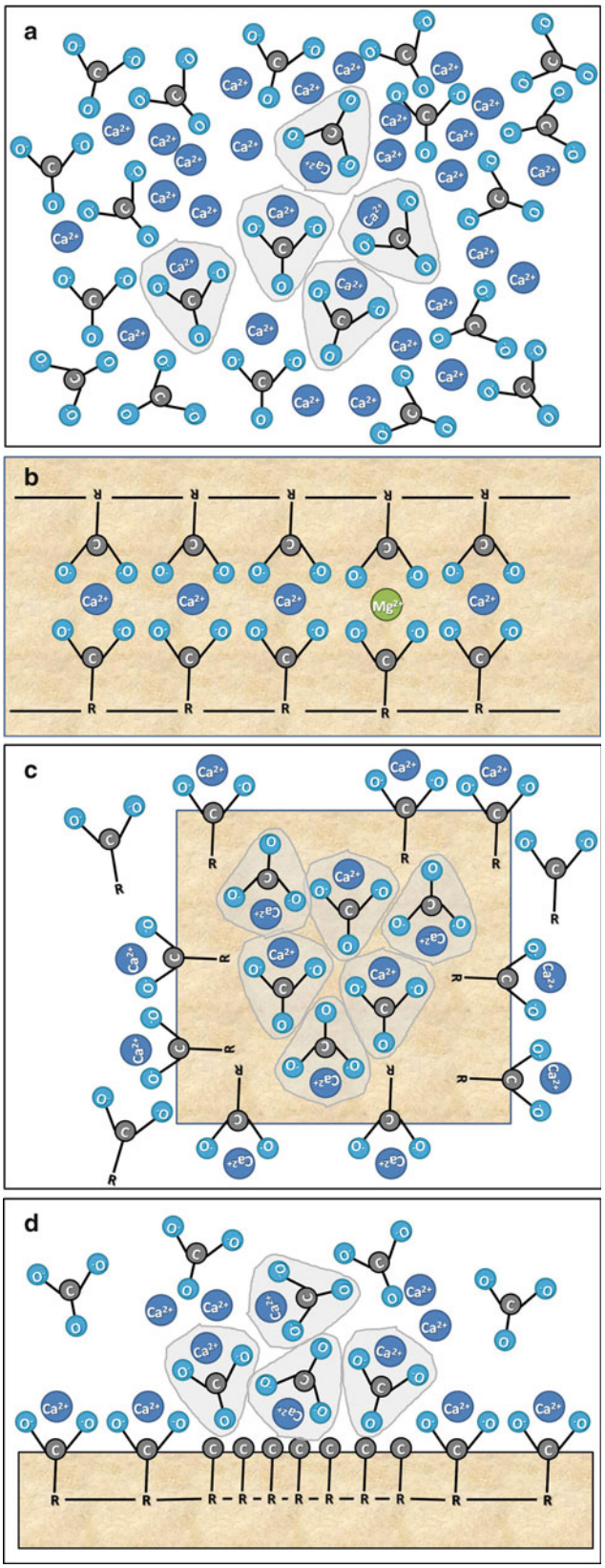

Fig. 4.16 Simplified model of the possible interactions of charged extracellular polymeric substances with calcium carbonate: (**a**) nucleation of calcium (Ca^{2+}) and carbonate (CO_3^{2-}) ions; (**b**) Inhibition of nucleation by a polyanion; (**c**) Inhibition of crystal growth by association of a crystallization nucleus with a polyanion; (**d**) Calcium carbonate crystal bound to a layer of charged polymers. The growth of the crystal may be arrested and the charged polymer may determine the eventual shape of the calcium carbonate crystal

where the organic matter is subject to degradation. Several others have observed the association of calcification with bacterial activity (Chafetz and Buczynski 1992; Krumbein 1979; Krumbein and Giele 1979).

Mucilage EPS is often produced by cyanobacteria as an overflow metabolism when they experience nutrient limitation. This is particularly the case under nitrogen depleted conditions, a situation common in the marine environment. In microbial mats, where extremely dense communities of cyanobacteria are present there is a high demand for nitrogen, while there is often a shortage of this important nutrient. Therefore, many microbial mats are diazotrophic, i.e. the cyanobacteria that build these mats fix atmospheric dinitrogen. However, most diazotrophic mats consist of non-heterocystous cyanobacteria. As argued in the section on N_2 fixation these cyanobacteria are not efficient N_2 fixers because the process is seriously hindered by oxygen. It is likely that such cyanobacteria in fact still are nitrogen limited. Cyanobacteria that grow under nitrogen limited conditions tend to produce a lot of mucilage (Ortega-Calvo and Stal 1994). It is possible that Precambrian calcifying microbial mats were not nitrogen-limited and that the growth of the organisms therefore might have been balanced with less overflow metabolism and mucilage production. This might also hold true for modern calcifying microbial mats. While some of these mats may rely on a sufficient external supply of combined nitrogen, others comprise heterocystous N_2-fixing cyanobacteria that satisfy their nitrogen demand. One example from freshwater environments is the Rivulariaceae. This group of heterocystous cyanobacteria produces extent calcium carbonate formations (Whitton 1987). The marine cyanobacterium *Calothrix* spp. belongs also to this taxonomic group and is known in some cases to form microbial mats but usually does not calcify.

A model for calcification and the development of stromatolites in the Exuma Cays, Bahamas is presented in Fig. 4.16. Subtidal and intertidal stromatolites that can be found in the Exuma Cays (Bahamas) are characterized by mats of the cyanobacterium *Schizothrix* sp. The model for calcification in these mats is based on a number of observations and assumptions. Two types of mats of *Schizothrix* can be distinguished. Lithifying microbial mats of *Schizothrix* are usually characterized by low ratios of photosynthesis over respiration while the opposite is true non-lithifying mats (Pinckney et al. 1995). Moreover, calcium carbonate was not associated with the cyanobacteria but with heterotrophic bacteria (Chafetz and Buczynski 1992; Chafetz 1994). It was further assumed that extracellular polymeric substances (EPS) may interfere by binding calcium and magnesium ions, thus locally inhibiting carbonate precipitation (Borman et al. 1982, 1987; Westbroek et al. 1994).

Exuma Cays stromatolites are formed at high energy sites. Intertidal stromatolites can be found on the Atlantic Ocean coast and are exposed to high wave energy. Subtidal stromatolites are almost exclusively encountered in channels with high currents. The cyanobacterium *Schizothrix* sp. is a filamentous organism composed of thin (often less than 1 μm wide) trichomes which are enveloped by a thin rigid polysaccharide sheath. This organism is capable of colonizing a solid substrate. Because grazing pressure will be low in these high-energy areas, a community of *Schizothrix* sp. may develop. Under conditions of low sedimentation rate a mat of *Schizothrix* sp. and associated microorganisms will develop (Fig. 4.17a). These mats are rigid and tightly associated with the underlying substrate. The cyanobacteria will grow and produce sheath material and possibly some mucus. It is assumed that this EPS will bind Ca^{2+} or that uronic acids prevent further growth of crystallization nuclei (Borman et al. 1982). During a period of sedimentation, *Schizothrix* sp. will move rapidly upwards by phototaxis and continue growth in the top layers where optimum light conditions prevail. The trichomes form a dense network in which carbonate sand grains (ooids) are trapped and agglutinated by EPS, while Ca^{2+} is further bound. Empty sheaths and other organic matter that has been abandoned deeper in the sediment will be decomposed (Fig. 4.17b). During a subsequent period of low rates of sedimentation a dense mat of *Schizothrix* sp. will develop in the top layer of the sediment. This layer is characterized by active growth of the cyanobacteria and may be associated with abundant production of EPS. The matrix of EPS in which the mat is embedded may bind Ca^{2+} efficiently and condenses EPS to the gel state (Rees 1969; Decho and Moriarty 1990; Decho 1994). It is conceived that this will lower the activity of this ion so that calcium carbonate will not precipitate. Depending on the physicochemical gradients that typically develop in microbial mats due to phototrophic and heterotrophic activities, some dissolution and re-precipitation of $CaCO_3$ and re-crystallization of the carbonate sediment grains may occur (Fig. 4.17c). During the next stage of development, *Schizothrix* sp. moves upward after another period of high rate of sedimentation. While growth of the cyanobacterium and the production of EPS in the new top layer trap and agglutinate the carbonate sand, the large amount organic matter that was left behind is decomposed by heterotrophic bacteria. Because EPS is also decomposed, Ca^{2+} that was bound will be released (Decho et al. 2005). This will locally result in supersaturation of calcium carbonate resulting in the formation of a microcrystalline crust of precipitated carbonate (Fig. 4.17d). A similar type of bacterial calcification occurs during the degradation of calcium oxalate. Oxalate is a product of metabolism of fungi and other organisms and is capable of immobilizing calcium (Verrecchia et al. 1990). In the Exuma Cay stromatolites another type of biologically influenced calcification occurs. The unicellular cyanobacterium *Solentia* bores in the calcium carbonate grains, dissolving them partly and the subsequent precipitation fuses the ooids, forming a solid lithified structure (Reid et al. 2000). This model explains that *in situ* calcium carbonate precipitation and lithification of the mat is

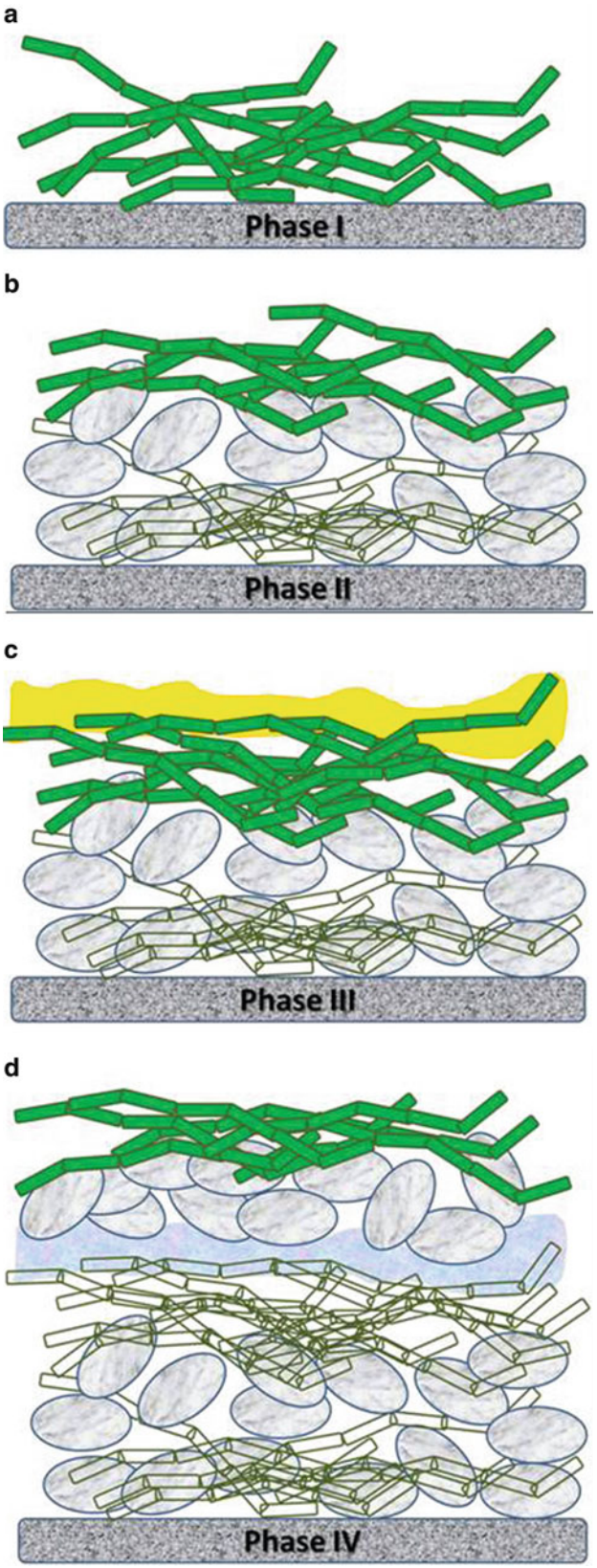

Fig. 4.17 Simplified scheme of the development of lithified micritic layers in Bahamas stromatolites. For explanation, see text

controlled by biology. It is indirectly associated with the cyanobacteria but degradation of organic matter, notably EPS, by heterotrophic bacteria is required for this process. Alternating periods with high and low rates of sedimentation are responsible for the formation of the laminated structure of the lithified microbial mats, which could therefore be termed stromatolites.

4.7 Nitrogen Metabolism and Nitrogen Fixation

4.7.1 Introduction

In cyanobacteria nitrogen content may amount up to about 10% of dry weight and is quantitatively the third most abundant element. Any shortage of it will immediately affect the amount of phycobiliproteins and, consequently, the efficiency of light harvesting for photosynthesis (Allen and Smith 1969). Cyanobacteria may produce a unique nitrogenous compound known as cyanophycin or multi-L-arginyl-poly(L-aspartic acid). Its high nitrogen content means that it can serve as a nitrogen reservoir (Mackerras et al. 1990a, b).

Cyanobacteria use a variety of nitrogen sources (Flores and Herrero 1994). Ammonia can be taken up by passive diffusion or the protonated form ammonium (NH_4^+) by a specific uptake system (Fig. 4.18). The amino acids arginine,

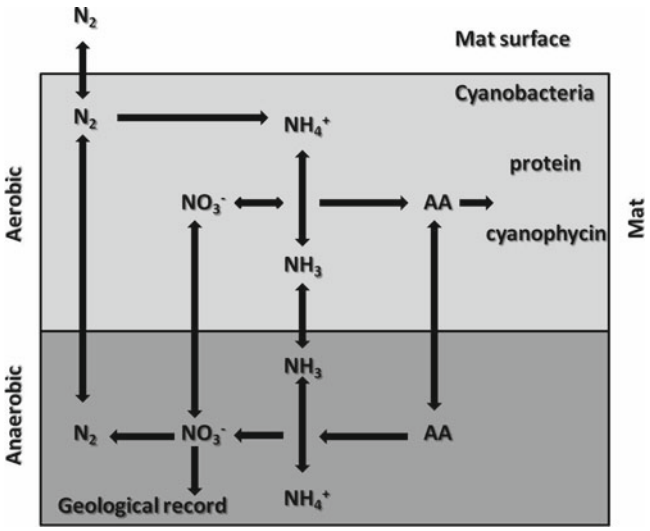

Fig. 4.18 The nitrogen cycle in a cyanobacterial mat. Cyanobacteria take up and assimilate ammonium into amino acids (*AA*) which are used for protein synthesis or can be stored as cyanophycin. Amino acids and ammonia may leak out the cells and oxidized to nitrate (nitrite) by nitrifying bacteria. In the anoxic part of the mat ammonium and nitrate (nitrite) is converted to dinitrogen by anammox and dinitrifying bacteria. Nitrate (nitrite) can be taken up by the cyanobacteria and assimilated. N_2-fixing cyanobacteria produce ammonium which is subsequently assimilated into cell material

asparagine and glutamine have been reported to serve as nitrogen sources in cyanobacteria (Flores and Herrero 1994). Nitrate and nitrite are important sources of nitrogen for cyanobacteria. This involves the uptake of nitrate or nitrite and its subsequent reduction to ammonia. This process involves ferredoxin as an electron donor and is therefore intimately associated with photosynthesis. Urea appears also to be a common nitrogen source for cyanobacteria (Moore et al. 2002; Valladares et al. 2002). Many cyanobacteria are capable of using dinitrogen (N_2) as the source of nitrogen.

4.7.2 The Nitrogen Cycle

Nitrogen occurs in different chemical oxidation states, varying from its most reduced form ammonia (NH_3) (−3), to hydroxylamine (NH_2OH) (−1), dinitrogen (N_2) (0), nitrous oxide (N_2O) (+1), nitric oxide (NO) (+2), nitrite (NO_2^-) (+3) to its most oxidized form nitrate (NO_3^-) (+5). All of these oxidation states are biologically significant and microorganisms may carry out reduction and oxidation reactions transforming one form into another. The element nitrogen therefore is subject to microbiological cycling in nature. In microbial mats all steps of the nitrogen cycle may be present and cyanobacteria play a particular important role (Fig. 4.17).

Ammonia is assimilated into amino acids that are used for the synthesis of proteins. Luxury uptake of nitrogen may occur and be stored as cyanophycin. Ammonia and amino acids may leak out of the cell. When oxygen is present, ammonium may be oxidized via nitrite to nitrate by nitrifying bacteria. Nitrate may be taken up by the cyanobacteria and assimilated or under anoxic conditions converted to gaseous nitrogen by denitrifying bacteria. Anaerobic ammonium oxidation (anammox) is another process that leads to the conversion of fixed nitrogen to N_2 (Jaeschke et al. 2009; Porubsky et al. 2009). Hence, these processes cause a loss of combined nitrogen, which is counteracted by the capacity of some cyanobacteria to fix dinitrogen (Joye and Paerl 1994). Nitrogen cycling in microbial mats contributes to the nutrient limitation patterns of mangrove trees. In dwarf habitats, microbial mats serve as a source of nitrogen via the fixation of dinitrogen, while in fringe and transition habitats, mats compete with the trees for nitrogen via denitrification (Lee and Joye 2006).

In microbial mats the decomposition of organic matter may be incomplete which could mean that part of the nitrogen is not recycled and so enters the geological record (Fig. 4.17). Hence this nitrogen is withdrawn from the microbial nitrogen cycle. It is not clear how important this process is since even in mats in which a net accretion of organic matter occurs, up to 99% of the produced organic matter may be recycled within the mat (Krumbein et al. 1977). In exceptional cases organic nitrogen produced by N_2-fixing cyanobacteria can be transformed to nitrate deposits known as guano or caliche nitrates (Ehrlich 1996).

Most of the nitrogen in the biosphere is present in the atmosphere in the form of dinitrogen (N_2), which amounts to 3.9×10^{18} kg N. In the oceans and on land the amount of combined nitrogen (organic and inorganic) amounts each about 10^{15} kg N. The amount of nitrogen in living biomass on earth amounts only 1.3×10^{13} kg (Ehrlich 1996). It is generally assumed that primary production in the marine environment is limited by nitrogen. In particular marine microbial mats with their dense and compressed biomass often experience a shortage of nitrogen. The majority of organisms cannot use the most abundant form of nitrogen, N_2. Only N_2-fixing organisms (diazotrophs) are capable of using dinitrogen. All these organisms possess nitrogenase. Cyanobacteria are among the most important diazotrophs and in all marine microbial mats that have been investigated to date, N_2 fixation has been observed.

4.7.3 Nitrogenase

In all N_2-fixing organisms the enzyme complex nitrogenase is present. This enzyme is similar in all organisms that contain it. The complex is composed of two enzymes: dinitrogenase reductase which is a dimer of identical subunits and also termed the iron-protein, which is encoded by *nifH*, and dinitrogenase, a tetramer composed of two different subunits (α_2 β_2), encoded by *nifDK*. Dinitrogenase is also known as the molybdenum-iron protein (Howard and Rees 1994).

Nitrogenase catalyzes the following reaction:

$$N_2 + 8e^- + 8H^+ + 16\,\text{MgATP} \rightarrow 2\,NH_3 + H_2 + 16\,\text{MgADP} + 16P_i$$

Reduced ferredoxin is the electron donor of nitrogenase. The equation shown above makes clear that the fixation of N_2 is at the expense of considerable amount of energy and low potential electrons. This high energy demand of nitrogenase presents often a problem for diazotrophic organisms except for cyanobacteria which produce reduced ferredoxin and convert light energy into chemical energy during photosynthesis. However, nitrogenase is extremely sensitive to oxygen and therefore diazotrophic organisms must provide an anaerobic environment in order to be able to fix N_2. Cyanobacteria as oxygenic phototrophic and principally aerobic organisms need special adaptations.

4.7.4 Dinitrogen-Fixing Cyanobacteria

Diazotrophic cyanobacteria are capable of using dinitrogen (N_2) as the sole source of nitrogen for growth. These organisms can be subdivided in three main groups (Table 4.3).

Table 4.3 Types and characteristics of N_2-fixing cyanobacteria

Type I Heterocystous cyanobacteria
Exclusively filamentous species that differentiate special cells: heterocysts
Strategy: spatial separation of N_2 fixation and oxygenic photosynthesis and protection of nitrogenase in the heterocyst
Diazotrophic growth under fully aerobic conditions
Examples: *Anabaena, Aphanizomenon, Calothrix, Fischerella, Mastigocladus, Nodularia, Nostoc, Scytonema*
Occurrence: waterblooms (freshwater lakes and brackish seas), paddy fields, various microbial mats, symbiotic with a variety of different organisms
Type II Anaerobic N_2-fixing non-heterocystous cyanobacteria
Filamentous and unicellular species
Strategy: avoidance (of oxygen)
Induction and maintenance of nitrogenase only under anoxia or low oxygen; sulhide may be necessary in order to inhibit oxygenic photosynthesis
Examples: *Geitlerinema, Leptolyngbya, Synechococcus*, many other cyanobacteria
Occurrence: many different environments, particularly microbial mats
Type III Aerobic N_2-fixing non-heterocystous cyanobacteria
Filamentous and unicellular species
Strategy: diverse and unknown (temporal separation of N_2 fixation and oxygenic photosynthesis in concert with oxygen protection mechanisms; or in case of *Trichodesmium* possibly a combination of temporal and spatial separation. Nitrogenase may or may not be confined to special cells termed 'diazocytes'; or lacking PS-II in uncultivated 'Group A')
Diazotrophic growth possible under fully aerobic conditions
Examples: *Crocosphaera*, *Cyanothece*, *Gloeothece*, *Lyngbya*, *Symploca*, *Synechococcus*, *Trichodesmium*
Occurrence: tropical ocean (*Crocosphaera, Cyanothece, Trichodesmium*), carbonate cave walls and paddy fields (*Gloeothece*), microbial mats (*Cyanothece*, *Gloeothece*, *Lyngbya*, *Symploca*, *Synechococcus*)

Group I consists of heterocystous cyanobacteria. These filamentous organisms differentiate special cells, heterocysts, which have lost the capacity of oxygenic photosynthesis and have evolved a modified thick cell envelope. Heterocysts are the site of N_2 fixation in these cyanobacteria. The thick cell wall contains special lipopolysaccharides and forms a diffusion barrier for gases, limiting the entry of oxygen. Respiration scavenges the little oxygen that enters the heterocyst. Since photosystem II is absent from the heterocyst, no photosynthetic oxygen is evolved by these cells. Therefore the heterocyst is virtually anoxic and provides an excellent environment for the oxygen-sensitive nitrogenase. Photosystem I-mediated conversion of light energy in the heterocyst provides nitrogenase with ATP. However, for reducing equivalents nitrogenase depends on the import of carbohydrates from the neighbouring vegetative cells. The strategy that heterocystous cyanobacteria have developed in order to be able to grow diazotrophically can be best described as the spatial separation of the two incompatible processes of N_2 fixation and oxygenic photosynthesis. Among the cyanobacteria heterocystous species are the ultimate adapted organisms for N_2 fixation. The vast majority of heterocystous cyanobacteria can be found in freshwater or terrestrial systems, both free-living and as symbionts.

Heterocystous cyanobacteria occur in some brackish basins but are rare in the marine environment, including microbial mats. *Fischerella* and *Mastigocladus* form mats in thermal springs. The heterocystous *Calothrix* sp. has been found as the dominant organism in microbial mats in the tidal area of the Pacific coast in Baja California Sur, Mexico. *Calothrix* is also known from a variety of other marine and brackish habitats such as the spray zone of rocky shores (Jones and Stewart 1969; Whitton and Potts 1982). Mats of the heterocystous cyanobacterium *Anabaena* have been found in a coastal lagoon in southwest France (Villbrandt and Stal 1996) and *Nodularia* occurs in coastal microbial mats of the Dutch barrier islands (Severin and Stal 2008). However, these are exceptions rather than a rule. The vast majority of microbial mats are built by non-heterocystous cyanobacteria, notwithstanding the facts that in many cases N_2 fixation is a crucial process in these systems.

Group II consists of filamentous and unicellular cyanobacteria that do not show cell differentiation and are capable of N_2 fixation only under virtually anoxic conditions with no oxygenic photosynthesis occurring. These organisms, although possessing the genetic capacity of synthesizing nitrogenase, have obviously not evolved a mechanism to protect effectively nitrogenase from oxygen inactivation. Consequently, their strategy can be characterized as avoidance of oxygenated environments. Such environments usually also prevent oxygenic photosynthesis. Among the non-heterocystous cyanobacteria up to about 50% may belong to this group of organisms but for virtually all of them it is uncertain whether they live diazotrophically in their natural environment. Non-heterocystous cyanobacteria that are capable of inducing nitrogenase activity under anaerobic conditions can be found in many environments, including microbial mats. However, most environments in which these cyanobacteria occur are permanently oxygenated and therefore diazotrophic growth is unlikely. In contrast, microbial mats are often characterized by steep and fluctuating gradients of oxygen and sulphide. Anoxia frequently occurs in microbial mats; this as a rule coincides with high levels of sulphide, a very potent inhibitor of oxygenic photosynthesis. Thus, it is not surprising that evidence hinted to anaerobic N_2-fixing cyanobacteria growing diazotrophically in microbial mats in which H_2S was present (Villbrandt and Stal 1996).

Group III cyanobacteria also comprise non-heterocystous filamentous and unicellular cyanobacteria but they are remarkable as they possess the capacity of inducing nitrogenase and growing diazotrophically under fully aerobic conditions. To date our knowledge of how these organisms are protecting their undoubtedly oxygen-sensitive nitrogenase

is incomplete. Although the number of species that we know that possess this capability is still relatively rare, their numbers are increasing at steady pace. Examples can be found in terrestrial environments such as cave-walls, paddy fields and thermal springs, and in the marine environment. Freshwater lakes apparently do not harbour aerobic N_2-fixing non-heterocystous cyanobacteria. In the ocean the planktonic colony-forming *Trichodesmium* spp. is known as an efficient diazotrophic growing, non-heterocystous cyanobacterium. In addition, several unicellular diazotrophic cyanobacteria occur in the oceans. These include *Crocosphaera* and *Cyanothece* (Needoba et al. 2007). The pico-sized and hitherto uncultivated 'Group A' cyanobacteria are abundant and metagenomic analyses suggest that these organisms may lack the oxygenic photosystem II (Zehr et al. 2008). Their mode of life is unclear and could either be photoheterotrophic or symbiotic. Strikingly, diazotrophic cyanobacteria in the oceans are confined to the tropical and subtropical regions with surface water temperature well above 20°C. In microbial mats aerobic N_2-fixing non-heterocystous cyanobacteria are reported to belong predominantly to the genera *Oscillatoria* and *Lyngbya*, which are morphologically and phylogenetically closely related to *Trichodesmium*. However, in a variety of environments unicellular N_2-fixing cyanobacteria such as *Cyanothece*, *Gloeothece* and *Synechococcus* are known to build microbial mats.

The first report of a culture of a filamentous non-heterocystous aerobic N_2-fixing cyanobacterium was by Pearson et al. (1979). This organism was originally identified as *Microcoleus chthonoplastes* but later renamed as *Symploca* sp. (Janson et al. 1998). *Symploca* sp. is also morphologically related to *Oscillatoria*, and was isolated from a tidal microbial mat (Pearson et al. 1979; Malin and Pearson 1988). It has been proposed that the strategy of aerobic non-heterocystous cyanobacteria, in analogy with the heterocystous species, is temporal separation of the incompatible processes of photosynthesis and N_2 fixation. The latter would than occur during the dark (Mullineaux et al. 1981; Stal and Krumbein 1987). However, not all species in this group follow this strategy. *Trichodesmium* spp. fix N_2 during the day (Capone et al. 1990). Moreover, all species that have been cultured are capable of growing diazotrophically under continuous light and, in the unicellular *Gloeothece* sp., culture conditions can be chosen under which N_2 fixation occurs during the light period of a light dark cycle (Ortega-Calvo and Stal 1991).

4.7.5 Daily Variation of N_2 Fixation

N_2 fixation has a high demand of energy and low-potential reducing equivalents. For the oxygenic phototrophic cyanobacteria light is the source of ATP generation and electrons are derived from water and transferred to ferredoxin mediated by photosynthetic electron transport. Thus, in cyanobacterial mats N_2 fixation ought to be directly linked to light. However, since oxygen exerts a negative effect on nitrogenase, daily variations of N_2 fixation in microbial mats can be expected. The patterns of these daily variations will depend on the type of diazotrophic cyanobacterium and on the dynamics of light and oxygen in the mat. In Fig. 4.19 five daily patterns of nitrogenase activity, measured in different microbial mats, are depicted.

N_2 fixation in heterocystous cyanobacteria is intimately linked to light. The heterocyst is not capable of CO_2 fixation and therefore does not accumulate storage carbohydrate, as is the case in vegetative cells. Dark energy generation in heterocysts will be limited because at one hand the reducing equivalents must be imported from the vegetative cells while at the other hand the oxygen entry in the heterocyst is limited as a result of the diffusion barrier provided by the cell wall (Walsby 1985). Therefore it is not surprising that daily variations of N_2 fixation in communities of heterocystous cyanobacteria are strongly light dependent (Griffiths et al. 1987; Storch et al. 1990; Stal 1995; Falcón et al. 2007) (Fig. 4.18a). However, considerable dark nitrogenase activity may occur in such communities. The ratio of light over dark nitrogenase activity in different populations of heterocystous cyanobacteria varies considerably and is possibly dependent on the species, the light history or other conditions.

In microbial mat communities composed of non-heterocystous cyanobacteria the daily pattern of N_2 fixation is less predictable (Paerl et al. 1989, 1996) (Fig. 4.18b–e). This depends largely on the type of organism and prevailing conditions in the mat. Moreover, due to the fact that these conditions may also vary from day to day (tidal movement, light and overcast, temperature and other factors), the daily pattern of N_2 fixation may change considerably.

The daily pattern of N_2 fixation in non-heterocystous cyanobacteria is the result of the combined effects of oxygen, light, and in some cases sulphide. As in heterocystous cyanobacteria, non-heterocystous species must supply nitrogenase with sufficient energy and low-potential reducing equivalents. This condition is satisfied in the light but the serious drawback is the evolution of oxygen. In such mats, photosynthesis obviously must occur at daytime and N_2 fixation is confined to the night (Fig. 4.18b). For instance this is the case in mats of *Gloeothece* and *Oscillatoria*. Whereas during the day microbial mats often become supersaturated with oxygen because the diffusion of this gas is limited, at night they may turn anoxic within minutes (Stal 1995). The microbial community, including the cyanobacteria, consumes oxygen in the dark by respiration. Obviously, anoxic conditions are ideal for N_2 fixation, but pose a problem with respect to the supply of energy and reducing equivalents. However, all cyanobacteria isolated from marine microbial mats and tested appeared to be

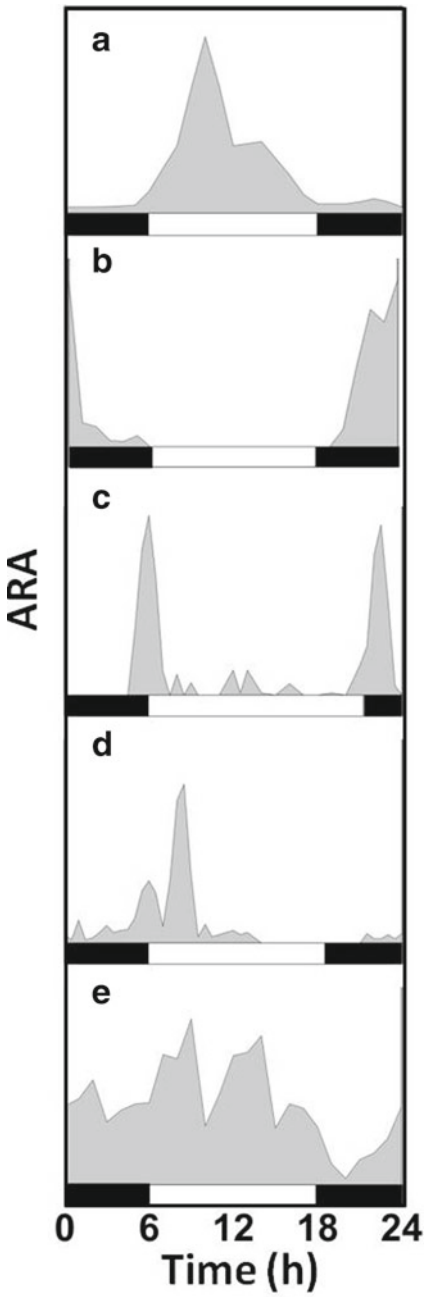

Fig. 4.19 Five typical patterns of daily variation of N_2 fixation (ARA, relative units) in microbial mats: (**a**) Mat of the heterocystous *Calothrix* sp. in Baja California, Mexico (Data from Stal et al. 1994); (**b**) Mat of the unicellular *Gloeothece* sp. on the wall of a carbonate cave (Data from Griffiths et al. 1987); (**c**) Mixed mat of the non-heterocystous *Microcoleus chthonoplastes* and *Lyngbya* sp. of a tidal flat of the North Sea island of Mellum (Data from Villbrandt et al. 1990); (**d**) Mat dominated by *Lyngbya* sp. (location as **c**) (Data from Villbrandt et al. 1990); (**e**) Mat of non-heterocystous *Lyngbya aestuarii* (location as **a**) (Author, unpublished)

capable of fermentation of endogenous storage carbohydrate (Stal and Moezelaar 1997). Although the energy generation by fermentation is undoubtedly small, it has been shown that it exceeds many times the extremely low maintenance requirements of cyanobacteria (Stal and Moezelaar 1997). It has also been shown that dark anoxic conditions supported considerable nitrogenase activity in the filamentous, non-heterocystous cyanobacterium *Oscillatoria limosa* (*Lyngbya aestuarii*) (Stal and Heyer 1987). In microbial mats in which this cyanobacterium occurred, daily patterns of N_2 fixation were found in which this activity was low but totally confined to the dark (Villbrandt et al. 1990). However, other patterns were also observed at different times in the same mats with the same organism. For instance, it could often be seen that nitrogenase activity peaked around sunset and sunrise (Fig. 4.18c). This was confirmed by experiments with *O. limosa* grown in the laboratory under an alternating light dark cycle and with anoxic conditions established 1 h after the onset of the dark period and aerobic conditions 1 h after the onset of the light period (Stal and Heyer 1987). Highest nitrogenase activities in these cultures were obtained in the light in the absence of oxygen. Also, in natural samples it has been observed that highest nitrogenase activities occurred at sunrise (Villbrandt et al. 1990) (Fig. 4.18d). Exactly the same observation was done for the hot spring unicellular cyanobacterium *Synechococcus* (Steunou et al. 2008). *NifH* was transcribed at the end of the day but the nitrogenase was present during the whole night and disappeared once the mat became enriched by oxygen the next day. Nevertheless, during most of the night nitrogenase activity was low, and revealed a small peak at sunset and a large peak at sunrise. This is because light is available while oxygen is still absent. After sunset, oxygen may have been present for some time, allowing energy generation through aerobic respiration. Once anoxic conditions are established only low rates of nitrogenase activity can be supported by the lower rate of fermentative energy generation. Vertical profiles of oxygen measured during a 24 h period have shown that the mat which possessed this type of fluctuating nitrogenase activity, oxygen was indeed present during the first hours after sunset and that it appeared again in the morning only hours after sunrise.

In freshly colonized sediments of North Sea tidal sand flats, *O. limosa* (*L. aestuarii*) is often the pioneer cyanobacterium (Stal et al. 1985). This is most likely because of its capacity to grow diazotrophically. In this pioneer state biomass is low and therefore so is the oxygen demand in the dark. Such sediments normally do not turn anoxic. However, during the light they may accumulate oxygen up to several fold saturation (Villbrandt et al. 1990). N_2 fixation in such systems is typically confined to the night, peaking at sunrise when light becomes available but at oxygen levels well below air saturation (Fig. 4.18d). In other systems such as in mats of the unicellular N_2-fixing *Gloeothece,* which grows on carbonate cave walls, a peak of nitrogenase activity is observed immediately after sunset and then decreasing gradually until hardly any activity is detectable at the end of the night

(Fig. 4.18b). This organism depends on oxygen for respiratory energy generation and it is possible that in the course of the dark period this organism depletes its endogenous storage carbohydrate (Maryan et al. 1986).

Another type of daily pattern of N_2 fixation in microbial mats of non-heterocystous cyanobacteria is more or less constant activity or fluctuations scattered throughout the day and night (Fig. 4.18e). This is often the case when Group 2 diazotrophic cyanobacteria are involved. These cyanobacteria are only capable of fixing N_2 under anoxic conditions or at least when oxygen concentrations are low and oxygenic photosynthesis is inhibited. Such a situation can be expected in microbial mats in which high concentrations of sulphide inhibit oxygenic photosynthesis. In the light, sulphide at the same time may serve as electron donor for nitrogenase in these situations. In most cases oxygenic photosynthesis is continuing in the surface layers of the mat. Sulphide in inhibitory concentrations for photosynthesis is usually present in the deeper layers where light intensity will also be low. In the dark, N_2 fixation may be supported by fermentation of endogenous storage carbohydrate. Thus both in the light and in the dark relatively low nitrogenase activities can be expected.

Severin and Stal (2008) recorded light-response curves of nitrogenase activity in coastal microbial mats. They observed changes in the fitted parameters of nitrogenase activity during a 24 h cycle and used these parameters and the monitored natural light intensities to calculate the daily amount of N_2 fixation. The daily variations of nitrogenase activity in the different types of microbial mats agreed with those that have been found previously and were typical for the cyanobacterial communities present in these mats (Fig. 4.20). Severin and Stal (2008) also integrated the daily amount of N_2 fixation during different days with different total daily irradiances and found that it was independent on the amount of light received by the microbial mat, even if nitrogenase activity had a strong light response. Also, the two types of microbial mats which were investigated and which were characterized by totally different daily patterns of nitrogenase activity, did not differ in their total daily integrated amount of N_2 fixation (Table 4.4). These authors concluded that N_2 fixation in these microbial mats was tuned to a maximum by the concerted action of a diverse diazotrophic community in which different components become active at different times as the result of the changing conditions.

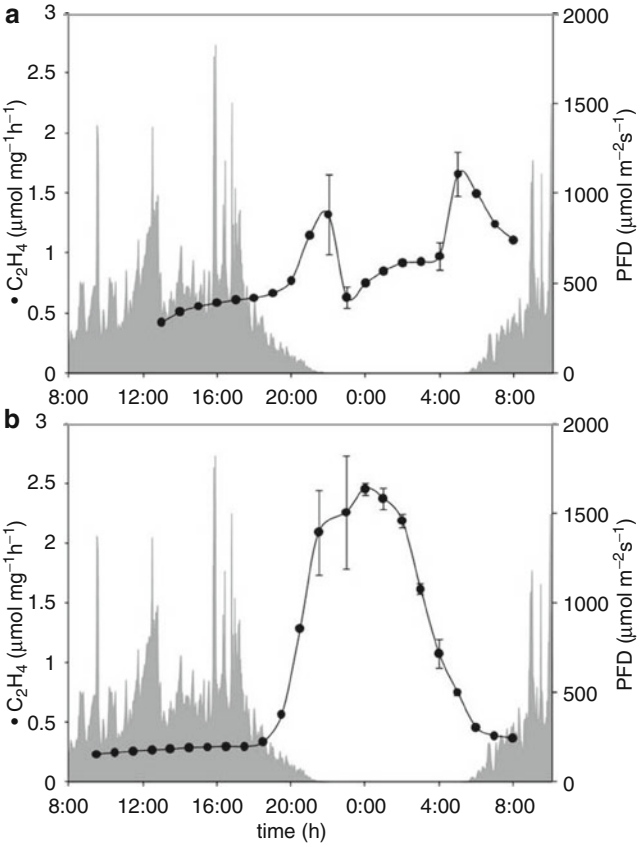

Fig. 4.20 **Daily patterns of N_2 fixation in two different types of microbial mat on the green beach of the North Sea island Schiermonnikoog:** (**a**) Mat containing a variety of filamentous cyanobacteria, including heterocystous species; (**b**) Mat containing predominantly *Lyngbya* sp. Nitrogenase activity (*circles*) was calculated from the actual ambient irradiance (*grey area*) and the light response curves (ARA) recorded for both mats at hourly intervals (From Severin and Stal 2008)

Table 4.4 Daily integrated nitrogenase activity (µmol C_2H_4 mg chl a^{-1}) and photon flux (µmol m^{-2}) for two microbial mats (After Severin and Stal 2008)

	Station I		Station II	
Date	Nitrogenase activity	Photon flux	Nitrogenase activity	Photon flux
28.05.06	17.9	26,295	20.4	23,993
29.05.06	18.0	29,399	17.5	29,275
30.05.06	17.7	23,866	17.5	29,443
31.05.06	17.8	15,839	17.5	14,052
Average	17.9		18.2	

4.7.6 Vertical Distribution of N_2 Fixation in Microbial Mats

Little is known about the vertical distribution of N_2 fixation in microbial mats, but the vertical distribution and dynamics of factors that control it such as light, oxygen and sulphide have been investigated in considerable detail. Light is attenuated strongly in microbial mats. The wavelengths that are absorbed by the cyanobacteria in the top layers are obviously attenuated most strongly. Far red light (<700 nm), however, is absorbed by the cyanobacteria to only a small extent; also the attenuation of this light in (wet) sediment is small compared to shorter wavelengths. Far red light does not support oxygenic

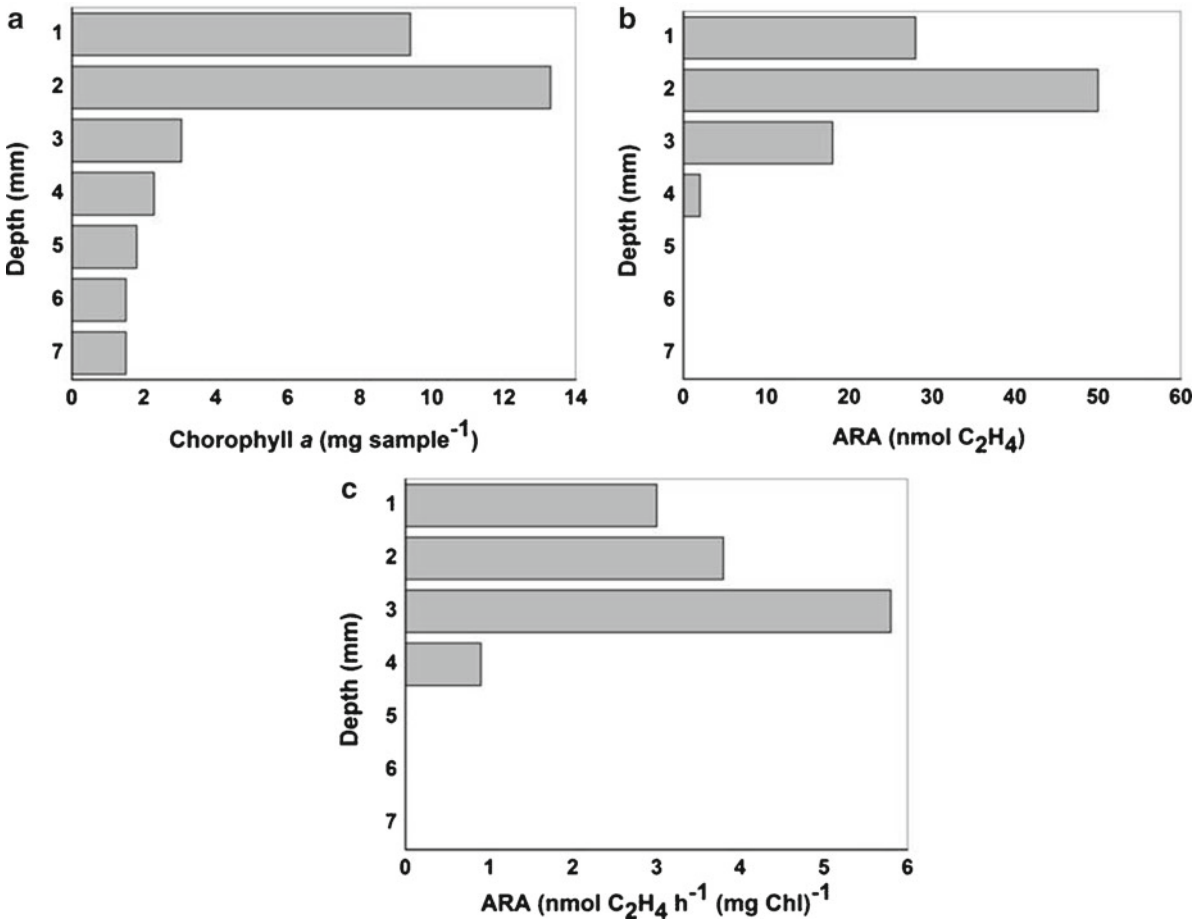

Fig. 4.21 Vertical distribution of chlorophyll *a* in: (**a**) potential nitrogenase activity (acetylene reduction, ARA); (**b**) specific, chlorophyll *a*-based ARA; (**c**) a microbial mat (Data from Stal et al. 1984)

photosynthesis but anoxygenic photosynthesis depends on it. It can thus be assumed that the cyanobacteria in the lower part of the mat are not capable of oxygenic photosynthesis. This has been shown by microelectrode measurements of oxygen concentration and photosynthesis. Such measurements have also shown that in some microbial mats sulphide is present in these layers. In an attempt to measure potential nitrogenase activity in microbial mats it was shown that in a mat of 3-mm maximum surface related nitrogenase activity occurred in the depth horizon of 1–2 mm (Stal et al. 1984). However, when nitrogenase activity was expressed on the basis of chlorophyll *a*, highest specific nitrogenase activity was present in the lowest layer of the cyanobacterial mat (2–3 mm) (Fig. 4.21). Cyanobacterial biomass was highest in the top layer, decreasing gradually until about 3 mm depth. Thus it is likely that a spatial separation of N_2 fixation and oxygenic photosynthesis had occurred in this mat. The top layer carries out oxygenic photosynthesis and CO_2 fixation, while N_2 is fixed in the lower layers.

4.7.7 Effects of Anoxia and Sulphide on N_2 Fixation in Microbial Mats

Among the non-heterocystous diazotrophic bacteria those that are capable of N_2 fixation under fully aerobic conditions are rare (Bergman et al. 1997). Since they grow by oxygenic photosynthesis, these organisms not normally perform N_2 fixation. It has been questioned whether this capacity of N_2 fixation is of any importance in the natural environment (Rippka and Waterbury 1977). Padan and Cohen (1982) mention that the facultative anoxygenic photosynthetic cyanobacterium *Oscillatoria limnetica* (*Geitlerinema*) is capable of N_2 fixation when carrying out sulphide-dependent anoxygenic photosynthesis. Villbrandt and Stal (1996) investigated the effect of sulphide on N_2 fixation in cyanobacterial mats and on cultures of cyanobacteria isolated from these mats. They compared a mat dominated by a heterocystous cyanobacterium (*Anabaena*) with another one dominated by non-heterocystous filamentous organisms (*Oscillatoria* and *Phormidium*). Sulphide inhibited

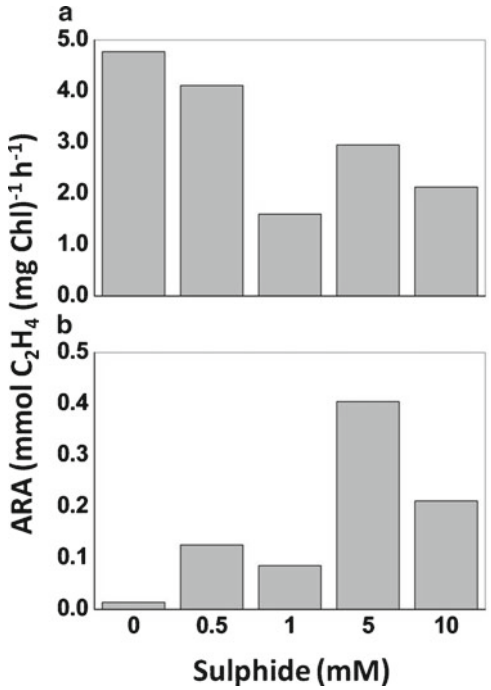

Fig. 4.22 Effect of sulphide on nitrogenase activity (acetylene reduction, ARA) in: (**a**) mat of the heterocystous *Anabaena* sp. (lagoon, Atlantic coast of France); (**b**) mat of non-heterocystous cyanobacteria (*Oscillatoria* sp. and *Phormidium* sp.) (lagoon, Mediterranean coast of France) (Data from Villbrandt and Stal 1996)

nitrogenase activity in the mat of *Anabaena*, but greatly stimulated it in the mat of non-heterocystous cyanobacteria (Fig. 4.22). Both light and dark nitrogenase activity was inhibited by sulphide in the mat of *Anabaena* but when DCMU was added in order to inhibit oxygenic photosynthesis virtually no effect of sulphide on N_2 fixation was seen in this mat. Therefore the effect of sulphide was mainly through the inhibition of oxygenic photosynthesis and respiration. Only the addition of 10 mM sulphide resulted in the almost complete inhibition of dark nitrogenase activity which depends on respiratory energy generation. In the light, this sulphide concentration resulted in a decrease of nitrogenase activity to the level obtained in the presence of DCMU, which in this case was about 40% of the control. This demonstrated that this amount of sulphide caused the complete inhibition of oxygenic photosynthesis. Due to the large amount of iron in this mat the actual concentration of free sulphide was probably much lower. Moreover, as was shown in a laboratory culture of *Anabaena* isolated from this mat, the effect of sulphide strongly depended on the pH. At pH 9.5 a total sulphide concentration of 5 mM had no effect on N_2 fixation but at pH 6.5 this concentration almost completely inhibited nitrogenase activity. This shows that the effect of sulphide is through the gaseous species H_2S. This gas will passively diffuse into the cell. Because the pH in these mats is usually high, very little H_2S will be present, even when the total concentration of sulphide is high.

In non-heterocystous mats the situation is totally different. In the control, without sulphide, nitrogenase activity is low in the light. In the dark or when oxygenic photosynthesis was inhibited by DCMU, nitrogenase activity was greatly stimulated. This can obviously be explained by the sensitivity of N_2 fixation in these organisms for photosynthetic and atmospheric oxygen. Sulphide stimulated nitrogenase activity in the light, in the dark and with DCMU. Stimulation was most marked in the light and reached a maximum at 5 mM (Fig. 4.21b). However, even at 10 mM sulphide nitrogenase activity was about tenfold the control. The stimulation in the dark was small (it doubled) and reached already maximum at 0.5 mM. Also, with DCMU, stimulation was maximal at 0.5 mM, the same order of magnitude as the effect in the dark (Villbrandt and Stal 1996). The effect of sulphide on the light activity of nitrogenase is best explained by its inhibition of oxygenic photosynthesis in concert with a lowering of environmental oxygen concentration.

None of the cyanobacteria isolated from this mat possessed the capacity for aerobic N_2 fixation, but all of the strains were capable of inducing nitrogenase under anaerobic conditions (Villbrandt and Stal 1996). In experiments with *Phormidium*, it was shown that sulphide (total concentration up to 8 mM) had no effect on nitrogenase activity when this was induced anaerobically with DCMU. Therefore it was concluded that sulphide did not act as an electron donor to nitrogenase and that the stimulatory effect observed in the mat was most probably due to the scavenging of environmental oxygen. Sulphide very efficiently induced nitrogenase in *Phormidium* and other non-heterocystous cyanobacteria with anaerobic nitrogenase. About 4 mM total sulphide was sufficient for full induction of nitrogenase. However, in contrast with what was seen with the heterocystous cyanobacterium and to what was expected, it appeared that induction of nitrogenase with sulphide was optimal at high pH, which means that the ions HS^- and/or S^{2-} were more efficient than the gas H_2S. This suggested that *Phormidium* could actively take up sulphide ion. Thus the uptake of sulphide ion may be essential to allow diazotrophic growth in these organisms.

4.7.8 Oxygen Protection of Nitrogenase in Microbial Mats

Because cyanobacterial mats often contain a high density of biomass and have low rates of molecular diffusion, they may become markedly supersaturated with oxygen. This poses the question of oxygen protection of nitrogenase in microbial mats. Although in heterocystous cyanobacteria nitrogenase is confined to the heterocysts, and protected from oxygen under normal atmospheric conditions, it has been shown that N_2 fixation may be seriously impaired at oxygen pressure well above atmospheric levels. In the majority of cases,

non-heterocystous cyanobacteria are the dominant organisms in mats. Many of these species possess only the capacity of anaerobic nitrogenase activity because the lack of an adequate oxygen protection mechanism. They may be able to grow diazotrophically under anaerobic conditions and when sulphide inhibits oxygenic photosynthesis. This may under circumstances lead to a vertical spatial separation of oxygenic photosynthesis in the top layer of the mat and N_2 fixation in the deeper parts. Paerl and Prufert (1987) and Paerl et al. (1995) emphasized the importance for N_2 fixation of anoxic microzones in microbial mats and other systems. In a few cases, microbial mats have been shown to be built by non-heterocystous cyanobacteria that are capable of N_2 fixation under fully aerobic conditions (Pearson et al. 1979; Stal et al. 1984; Villbrandt et al. 1990; Gallon et al. 1991; Paerl et al. 1991). Since nitrogenase in these organisms is as sensitive to oxygen as in any other organism, these cyanobacteria obviously must possess a protection mechanism. Despite a large amount of research on this problem the precise mechanism by which these species protect nitrogenase from oxygen inactivation is still not known (Bergman et al. 1997). In fact, in all cases in which aerobic N_2-fixing cyanobacteria form microbial mats, N_2 fixation is confined to the night. Thus, a temporal separation of N_2 fixation and photosynthesis (respectively during the night and during the day) is maintained (Stal 1995). Because these mats turn anoxic during the night, there is no need for oxygen protection. The problem of oxygen protection of nitrogenase in microbial mats is therefore hardly relevant.

Aerobic N_2-fixing non-heterocystous cyanobacteria isolated from microbial mats include the filamentous *Oscillatoria*, *Lyngbya*, and *Microcoleus*, and the unicellular *Gloeothece*, *Cyanothece* and *Synechococcus* (Bergman et al. 1997). Among the different mechanisms that have been proposed for oxygen protection of nitrogenase, the uptake and reduction of oxygen, seems to be the most promising. Such systems may act in concert with enzymes that remove oxygen radicals.

In Table 4.5 the effects of different treatments of a diazotrophic microbial mat composed of *Oscillatoria* on nitrogenase activity are shown. When these mats were incubated in the laboratory and exposed to elevated salinity, phosphate fertilization or to a tidal movement of the water, all these treatments resulted in a dramatic increase of nitrogenase activity. The application of a tidal movement (alternating immersion and emersion of the mat) resulted in a two orders of magnitude increase in nitrogenase activity. The vertical profiles of oxygen in these mats, measured at the same time, showed that the increase of N_2 fixation was probably the result of markedly decreased concentrations of oxygen. The reference showed oxygen supersaturation, peaking at about 250 μm depth, typical for these mats. When the mats were subject to increased salinity, phosphate fertilization or to a tidal movement, oxygen profiles decreased dramatically. The decrease of oxygen concentration was most pronounced in the mats subject to both phosphate fertilization and tidal movement. These treatments also resulted in the strongest stimulation of nitrogenase activity. It is obvious that the decreased oxygen concentration is associated with the increased potential to fix dinitrogen.

Table 4.5 Effect of different treatments of a nitrogen-fixing microbial mat of *Oscillatoria* sp. from the island of Texel, The Netherlands

Treatment	Nitrogenase activity (nmol C_2H_4 cm^{-2} h^{-1})
Reference	2 ± 1
High salinity	31 ± 5
Phosphate fertilization	164 ± 18
Tidal movement	234 ± 21

Sediment cores containing the mat were incubated in the laboratory in aquaria filled with seawater (Instant Ocean). The seawater was aerated. Illumination was by 75 W halogen lamps applied at a 16–8 h light–dark cycle. Heating of the mats was prevented by a heat filter and fans. The reference cores were incubated in such a way that the mat was just exposed while the water level was just underneath the mat surface. The mat surface was moist. This incubation mimics the natural situation most closely. In another aquarium the seawater was pumped in and out at 6-h intervals, mimicking a tidal movement. At each high water the mat was covered by 5 cm of water. The tidal range was about 15 cm. In the third incubation the salinity was increased to twice the normal value (3%). In the fourth treatment, phosphate concentration in the seawater was increased to 100 μM. The cores were incubated for 1 week and vertical oxygen profiles and nitrogenase activity (acetylene reduction) were measured

One possibility that must be investigated is the capacity of nitrogenase in these cyanobacteria to reduce oxygen (autoprotection) (Bergman et al. 1997). This causes the reduction of O_2 to H_2O_2, which can be further reduced by peroxidases.

4.7.9 Heterocystous Versus Non-heterocystous Cyanobacteria in Microbial Mats

There is no doubt that heterocystous cyanobacteria are particularly well adapted for diazotrophic growth. They can fix N_2 in the light while carrying out oxygenic photosynthesis. In this way they make optimal use of light energy to satisfy the large demands of nitrogenase. Oxygen protection of nitrogenase in these organisms is assured by the heterocyst. Anoxic conditions usually result in scarcely higher nitrogenase activities and the inhibition of oxygenic photosynthesis by DCMU invariably results in lower activities, apparently because it inhibits the flow of reduction equivalents from the vegetative cells. Non-heterocystous cyanobacteria either cannot fix N_2 at all in the presence of oxygen or those that can invariably can much better in the dark or when transferred to anoxic conditions (Stal 1995). Often the inhibition of oxygenic photosynthesis by DCMU also stimulates nitrogenase

activity considerably. Notwithstanding these facts, the vast majority of marine microbial mats are composed of non-heterocystous cyanobacteria. Thus the question is raised as to why heterocystous cyanobacteria are not more common in these mats.

On the tidal flats in Guerrero Negro, Baja California Sur, Mexico, two types of microbial mats can be found in close vicinity of each other (Stal et al. 1994; Stal 1995) (Fig. 4.7). The smooth mat is composed of the non-heterocystous cyanobacterium *Lyngbya aestuarii* and covers the lower areas of the tidal flat. On the upper tidal flat and on slightly elevated spots a pustular mat develops which is composed of the heterocystous cyanobacterium *Calothrix*. Both mats fix N_2 but show distinct differences in their daily nitrogenase patterns. *Calothrix* fixes predominantly during the day while nitrogenase activity in the mats of *L. aestuarii* is confined to the night. Due to their locations on the tidal flat the mats of *L. aestuarii* are covered more often and during longer periods of time at high tide than the mats of *Calothrix*. During inundation of the mats of *L. aestuarri* diffusion is limited. This causes oxygen supersaturation during the period of photosynthesis and anoxic conditions at night. These anoxic conditions also allow the development of a community of sulphate-reducing bacteria. This mat has a very dense biomass and is characterized by steep gradients of oxygen and sulphide, typical for microbial mats. Due to this dense mat structure the gradients of oxygen and sulphide exist, regardless whether the mat is inundated or not.

The situation in the mat of *Calothrix* is totally different. This pustular mat has a porous structure. Due to this structure in this mat there is a free exchange of oxygen with the atmosphere and oxygen supersaturation or anoxic conditions are not usually the case. In exceptional cases when the mat is inundated for a prolonged period of time anoxic conditions or oxygen supersaturation may occur but normally the mat will be inundated for short periods or not at all. Stal et al. (1994) hypothesized that heterocystous cyanobacteria would not be able to maintain themselves in an environment in which either dark anoxic conditions or high concentrations of sulphide occur. Cyanobacteria incapable of fermentation will die within 2–3 h of dark anoxic conditions (Stal and Moezelaar 1997). However, there is no reason why heterocystous cyanobacteria should be incapable of fermentation and this has been demonstrated in a number of symbiotic *Nostoc* spp. (Margheri and Allotta 1993; De Philippis et al. 1996).

In order to investigate the possibility of sulphide as a selecting factor, Villbrandt and Stal (1996) compared a heterocystous and a non-heterocystous N_2-fixing mat in two coastal lagoons in France. The mat of heterocystous cyanobacteria was found in a lagoon with exceptional high amounts of iron, while this was not the case in the other system (Stal et al. 1996). As a result of the high amount of iron the sediment on which this microbial mat was found it did not contain any free sulphide because it precipitated as iron sulphide (Schaub and Van Gemerden 1996). Villbrandt and Stal (1996) hypothesized that the absence of sulphide would allow the proliferation of heterocystous species. On the one hand it was indeed demonstrated that N_2 fixation in heterocystous cyanobacteria was sensitive to sulphide. But on the other hand, unrealistic high concentrations of sulphide (10 mM) were required to obtain full inhibition. Sulphide inhibition of nitrogenase in heterocystous cyanobacteria depended on H_2S, which in microbial mats is present in very low concentrations as a result of the alkaline conditions. However, in heterocystous cyanobacteria it is uncertain whether other metabolic processes than N_2 fixation are more severely influenced by sulphide. In microbial mats and living stromatolites from Cuatro Cienegas, Mexico, evidence for the presence of heterocystous N_2-fixing cyanobacteria was obtained from the pattern of nitrogenase activity with highest activities during the day and its inhibition of oxygenic photosynthesis (Falcón et al. 2007). The negative effect of molybdate addition on nitrogenase activity observed by these authors was interpreted as a contribution of sulphate reducing bacteria to N_2 fixation. Molybdate inhibits sulphate reduction and this would therefore decrease the sulphide production. The ecological effects of such experiments are complex and difficult to interpret.

Another reason why heterocystous cyanobacteria are absent from the majority of microbial mats could lie in the fact that such organisms generally are not motile and that the link between the heterocyst and the vegetative cell is weak (Stal et al. 1994). Non-heterocystous cyanobacteria that form microbial mats are mostly motile by gliding movement. This is an important property since it facilitates optimal vertical positioning. It allows the cyanobacteria to compensate for the rapidly shifting physicochemical gradients in microbial mats. Gliding motility is also important because microbial mats often develop in environments that are characterized by high rates of sedimentation. In the rare cases that heterocystous cyanobacteria dominate microbial mats their filaments are orientated in a uniform manner at the mat surface. The tapered trichomes of *Calothrix* are orientated vertically with the terminal heterocysts situated away from the surface. These filaments do not glide freely. The same is the case for another mat-building heterocystous cyanobacterium, *Scytonema*, which likewise reveals a vertical orientation. In this organism intercalary heterocysts are formed located in the centre of the aggregates. It is likely that shear forces produced during gliding in these highly compressed microbial mats would result in the breakage of the weak link between heterocysts and the neighbouring vegetative cells. Hence, although gliding motility is an essential property for cyanobacteria in microbial mats, it has at the same time a serious disadvantage for heterocystous species. It is therefore

expected that mats of such cyanobacteria can only develop in environments with low rates of sedimentation and relatively constant physicochemical gradients.

4.7.10 Other Diazotrophic Organisms in Microbial Mats and the Case of *Microcoleus chthonoplastes*

The capacity of N_2 fixation is widespread among bacteria and archaea and such organisms are among those that form the microbial community of microbial mats. The question is therefore relevant whether or not microorganisms other than cyanobacteria contribute to the fixation of N_2 in microbial mats and to what extent. Chemotrophic bacteria and archaea will be confronted with a limited supply of substrate to satisfy the energy demand of nitrogenase. Although they do not evolve O_2 they will still have to cope with an aerobic environment and oxygen supersaturation as the result of the oxygenic photosynthesis of the cyanobacteria. Or they avoid the aerobic environment but this would limit their energy generation capabilities.

Anoxygenic phototrophs will have ample energy (sunlight) but may be limited in their sources of electron donor (i.e. sulphide or sulphur, ferrous iron, organic compounds). The problem of non-heterocystous cyanobacteria is to provide an anoxic environment for nitrogenase. Steppe et al. (1996) proposed a joint venture between diazotrophic bacteria and cyanobacteria. The latter would provide the former with organic matter and oxygen and the bacteria provide CO_2 and fixed nitrogen to the cyanobacteria. This model evolved from the observation that cultures and natural samples of the common and cosmopolitan mat-building cyanobacterium *Microcoleus chthonoplastes* possessed nitrogenase genes belonging to the γ- or δ-*Proteobacteria*, while cyanobacterial *nif* genes were lacking.

Although in the literature *M. chthonoplastes* has repeatedly been presented as a diazotroph, this may have been due to wrong identification (Garcia-Pichel et al. 1996; Siegesmund et al. 2008). For instance, the aerobically N_2-fixing *M. chthonoplastes* isolated by Pearson (Pearson et al. 1979; Malin and Pearson 1988) was later identified as *Symploca* sp. (Janson et al. 1998) and the anaerobic N_2-fixing *M. chthonoplastes* 'strain 11' is related to *Geitlerinema* (Siegesmund et al. 2008) to which genus also the Solar Lake strain '*Oscillatoria limnetica*' belongs. Dubinin et al. (1992) and Sroga (1997) reported also on N_2-fixing *Microcoleus* but their correct assignments await confirmation. Rippka et al. (1979) were unable to detect nitrogenase activity in the type strain of *M. chthonoplastes* PCC7420 and Villbrandt and Stal (unpublished results) were unable to induce nitrogenase activity under strictly anaerobic conditions in the collection of the 'true' *M. chthonoplastes* (Garcia-Pichel et al. 1996).

Bolhuis et al. (2010) discovered that a collection of *M. chthonoplastes* from distant geographic locations possess the structural genes for nitrogenase (*nifHDK*), but that they were not typical cyanobacterial but rather belong to the δ-Proteobacteria. The type strain of *M. chthonoplastes* PCC7420 possesses a full nitrogenase operon. These authors were unable to express the nitrogenase genes in any of these strains, though they showed expression in a microbial mat, indicating that the laboratoy conditions used were inappropriate for expressing nitrogenase in this strain. It was conceived that *M. chthonoplastes* obtained the nitrogenase operon from a sulphate reducing bacterium through lateral gene transfer. Hence, the attribution of N_2 fixation to organisms other than cyanobacteria may have been erroneous in a number of reports.

Severin et al. (2010) showed that filamentous and unicellular cyanobacteria dominated the clone libraries of *nifH* and their transcripts in two coastal microbial mats, but that δ- and γ-Proteobacteria also contributed importantly. The *nifH* of the γ-Proteobacteria belonged predominantly to anoxygenic phototrophic purple sulphur bacteria. The *nifH* of the δ-Proteobacteria belonged partly to *M. chthonoplastes* and for the other part might have belonged to sulphate reducing bacteria. Other reports also mention the predominance of *nifH* belonging to δ- and γ-Proteobacteria (Zehr et al. 1995; Olson et al. 1999; Omoregie et al. 2004) in microbial mats and Steppe and Paerl (2002) showed the transcription of δ-proteobacterial *nifH*.

4.8 Cyanobacteria and the Sulphur Cycle in Microbial Mats

The sulphur cycle has a large impact on microbial mats either when sulphate is present and the end-oxidation of organic matter is carried out by sulphate reducing bacteria, or when the ecosystem receives primary sulphide as is the case in sulphur springs. Seawater contains abundant sulphate (28 mM) and therefore sulphate reduction is usually a dominant process in coastal and hypersaline microbial mats. Sulphate-reducing bacteria are essentially anaerobic micro-organisms that oxidize simple organic compounds using sulphate as electron acceptor, which results in the formation of sulphide. A variety of different chemotrophic microorganisms as well as cyanobacteria and purple sulphur bacteria are capable of reducing elemental sulphur to sulphide. Sulphide is eventually oxidized back to sulphate. This can be done anaerobically by anoxygenic phototrophic bacteria such as purple and green sulphur bacteria or also by some cyanobacteria. Elemental sulphur is produced as an intermediate in this process. The cycling between elemental sulphur (S_0) and sulphide (S^{2-}) is also called the 'mini sulphur cycle' and is probably a dominant process in microbial mats (Van Gemerden 1993).

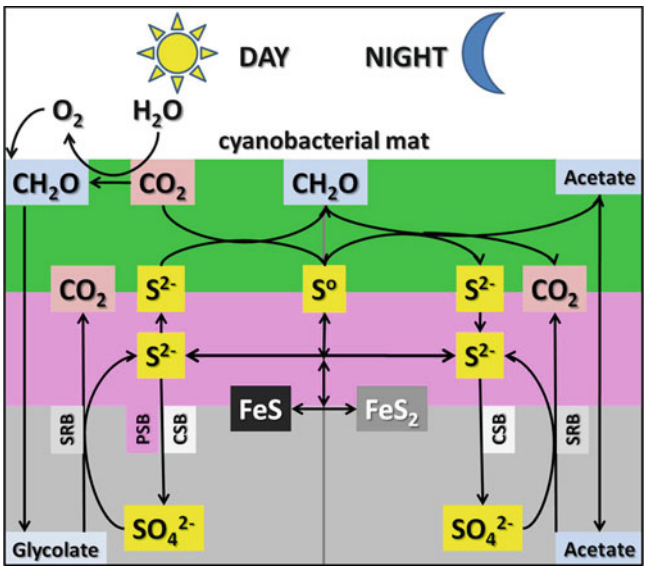

Fig. 4.23 A simplified scheme showing the role of cyanobacteria in the cycle of sulphur in a microbial mat. *SRB* sulphate-reducing bacteria, *CSB* colourless sulphur bacteria, *PSB* photosynthetic sulphur bacteria. For further explanation, see text

Sulphide-dependent anoxygenic photosynthesis by purple sulphur bacteria may account for more than 25% of the total photosynthetic carbon fixation as was found in microbial mats in the Ebro Delta in Spain (Martínez-Alonso et al. 2004). Some cyanobacteria are capable of sulphide-dependent anoxygenic photosynthesis, but oxidize sulphide only to elemental sulphur or to thiosulphate (Fig. 4.23). Colourless sulphur bacteria oxidize sulphide aerobically to sulphate but some species can carry out this oxidation anaerobically using nitrate as electron acceptor (denitrification). This anaerobic chemotrophic sulphide oxidation is probably not important because of the limited availability of nitrate in microbial mats. Hence, colourless sulphur bacteria and anoxygenic phototrophic bacteria compete for sulphide.

De Wit et al. (1995), using a mathematical model of a microbial mat, discovered a strikingly clear interaction between purple and colourless sulphur bacteria. Depending on the environmental parameters the model predicted that either the colourless sulphur bacteria dominate or that they coexist with purple sulphur bacteria. In the latter case purple sulphur bacteria can outweigh the colourless sulphur bacteria by more than an order of magnitude. This may explain why coastal or hypersaline microbial mats sometimes exhibit a purple layer and sometimes not, even when the black layer of FeS is present in both cases, pointing at anaerobic conditions and the presence and production of sulphide.

Another process that consumes considerable amounts of sulphide is when it reacts with ferric iron producing elemental sulphur. A peak of elemental sulphur has been observed underneath the aerobic zone where high rates of sulphate reduction occurred while sulphide remained very low due to oxidation by anoxygenic photosynthesis or by ferric iron (Wieland et al. 2005). Other processes in the sulphur cycle in microbial mats include the disproportionation of thiosulphate, sulphite and elemental sulphur. In these reactions one part of the molecule is oxidized while the other is reduced (Bak and Pfennig 1987; Canfield and Thamdrup 1996). The biomass of the major functional groups of microorganisms involved in the sulphur cycle, the purple sulphur bacteria, colourless sulphur bacteria and sulphate reducing bacteria may account for 40% of the total bacterial community (Visscher and Van Gemerden 1993).

In microbial mats most of the organic matter produced by photosynthetic CO_2 fixation is recycled. The organic matter (dissolved organic matter, DOM) is liberated into the mat environment by a variety of different mechanisms. Fermentation by the cyanobacteria results in the excretion of low-molecular organic carbon compounds (acetate, ethanol, and lactate) that serve directly as substrate for sulphate reducing bacteria. Photorespiration by cyanobacteria results in the formation and excretion of glycolate, which has also been shown to be used by sulphate-reducing bacteria (Fründ and Cohen 1992; Friedrich and Schink 1995) (Fig. 4.22). Degradation of more complex DOM, which is produced as a result of cell lysis or the exudation of extracellular polymeric substances (EPS), requires the combined action of several different microorganisms until it is eventually end-oxidized by sulphate reducing bacteria. Hence, the metabolic activity of the mat cyanobacteria can directly influence sulphate reduction.

It has been assumed that sulphate-reducing bacteria are present only below the euphotic depth in the microbial mat because only there, conditions are permanently anoxic. This layer is recognized by its black colour that indicates the presence of FeS. There are several lines of evidence that this may be incorrect. Sulphate reduction itself may take place under oxygenated conditions although the majority of sulphate-reducing bacteria are obligate anaerobes (Canfield and Des Marais 1991). However, sulphate-reducing bacteria are found throughout the microbial mat as is the case with sulphate reduction (Visscher et al. 1992; Stal 1993). In fact, 16S rRNA gene sequence analysis of microbial mats have demonstrated that the oxygen tolerant sulphate-reducing bacteria are predominantly found in the top layers of the mat while the obligate anaerobic species are found in the deeper layers of the mat (Risatti et al. 1994). Thus a vertical stratification of different groups of sulphate-reducing bacteria is likely and those in the top layer co-exist with cyanobacteria. Several reports have demonstrated the intimate association of cyanobacteria and sulphate reducing bacteria (Baumgartner et al. 2006). Throughout the hypersaline cyanobacterial mat of Solar Lake (Sinai, Egypt) sulphate-reducing bacteria were present and the rates of sulphate

Table 4.6 Groups of cyanobacteria with different types of adaptation to sulphide (After Cohen et al. 1986; Stal 1995)

Group 1.	Sulphide-sensitive oxygenic photosynthesis only
Group 2.	Sulphide-resistant oxygenic photosynthesis only
Group 3.	Sulphide-insensitive oxygenic photosynthesis concurrent with sulphide-dependent anoxygenic photosynthesis
Group 4.	Sulphide-sensitive oxygenic photosynthesis replaced by sulphide-dependent anoxygenic photosynthesis

reduction were sometimes higher in the oxygenated layer than in the deeper permanent anoxic parts of the mat (Teske et al. 1998). The dominant filamentous sulphate-reducing *Desulfonema* migrated during a day night cycle moving from the cyanobacterial layer and probably following the oxygen chemocline (Minz et al. 1999). This showed that some sulphate reducing bacteria tolerate substantial levels of oxygen. Facultative aerobic respiration and motility were considered as essential adaptations for these sulphate reducing bacteria to thrive in a microbial mat.

Many sulphate-reducing bacteria are much less oxygen-sensitive than had previously been assumed and sulphate-reducing bacteria are known that are even capable of aerobic respiration (Cypionka et al. 1985; Dilling and Cypionka 1990; Marschall et al. 1993). However, cultures that carry out dissimilatory sulphate reduction in the presence of oxygen have not been isolated thus far. It is possible that sulphate reduction in the oxygenated part of the microbial mat occurs in anoxic microniches, e.g. in aggregates.

Sulphate reduction also plays a crucial role in calcium carbonate precipitation and thereby controls the lithification process in stromatolites. This is supposed to be the result of two intertwined processes. First, the degradation of EPS liberates calcium that was bound to it and the sulphate reducing bacteria produce carbonate and raise the pH (Visscher et al. 2000; Dupraz et al. 2004).

Cohen et al. (1986) distinguished four groups of cyanobacteria with respect to the degree of sulphide inhibition and the possibility to carry out sulphide-dependent anoxygenic photosynthesis (Table 4.6). Cyanobacteria belonging to Group 1 are extremely sulphide sensitive. Oxygenic photosynthesis is inhibited at low levels of sulphide (<0.1 mM) and these species are not capable of anoxygenic photosynthesis. Cyanobacteria belonging to this group are evidently not important in marine microbial mats but are likely to be found in freshwater lakes, in the oceans or in terrestrial systems in which sulphide is absent or present at insignificant concentrations. Examples of such cyanobacteria are *Anacystis nidulans* (*Synechococcus elongatus*) and *Plectonema boryanum* (*Leptolyngbya boryana*) in which CO_2 fixation was inhibited at 60 and 75 μM (Cohen et al. 1986). In Group 2 cyanobacteria are represented that are incapable of anoxygenic photosynthesis but that resist considerable levels of sulphide. Oxygenic photosynthesis in these organisms is often stimulated at moderate (<1 mM) sulphide concentration. This type of adaptation is typical for marine microbial mats with fluctuating sulphide concentrations. The mat-forming and diazotrophic cyanobacterium *Oscillatoria limosa* (*Lyngbya aestuarii*) is a typical example of this group (Stal 1995). Also Group 3 cyanobacteria are typically found in marine microbial mats. These cyanobacteria are characterized by sulphide-insensitive oxygenic photosynthesis concurrent with sulphide-dependent anoxygenic photosynthesis. The cosmopolitan mat-forming cyanobacterium '*Microcoleus chthonoplastes*' (*Geitlerinema*) belongs to this group (De Wit and Van Gemerden 1988). Oxygenic photosynthesis in Group 4 cyanobacteria is as sensitive to sulphide as in those belonging to Group 1. The difference is in their capacity of carrying out sulphide-dependent anoxygenic photosynthesis. The sulphide tolerance of this group of cyanobacteria varies considerably from less than 1–10 mM. *Oscillatoria limnetica* (*Geitlerinema*) is the best-studied cyanobacterium belonging to that group. Photosystem II in this cyanobacterium is switched off when exposed to <0.1 mM of sulphide. Anoxygenic photosynthesis is induced in a process requiring protein synthesis. *O. limnetica* tolerates up to 9.5 mM sulphide but anoxygenic photosynthesis is gradually inhibited at concentrations exceeding 4 mM. A good example of how a mat community is structured with respect to sulphide was presented for microbial mats in Fuente Podrida, a cold sulphur spring located in East Spain (Camacho et al. 2005). Three filamentous cyanobacteria were found that fitted Group 1 (sensitive) UVFP3, in areas where sulphide was absent and Group 2 (tolerant) UVFP2 and Group 3 (anoxygenic photosynthesis) UVFP1. The latter isolate was related to *Planktothrix*, while the other cyanobacteria did not have close relatives. *Oscillatoria boryana* is also a typical Group 4 organism. This organism employs sulphide-dependent anoxygenic photosynthesis in the early morning which depleted sulphide locally (Castenholz et al. 1991). Sulphide concentrations of over 1 mM inhibit oxygenic photosynthesis completely. With increasing light intensity oxygenic photosynthesis became dominant. At low light sulphide-dependent anoxygenic photosynthesis remained the dominant mode. *O. boryana* is also able to photosynthesize at substantial rates over a wide range of sulphide concentrations by shifting between oxygenic and anoxygenic modes and possibly by combining both.

In microbial mats most of the sulphide is present as 'acid-volatile sulphide' (AVS) which is mostly in the form of ferrous sulphide (FeS) (Fig. 4.22). In this form sulphide is virtually insoluble. Only free sulphide may be toxic. Free dissolved sulphide occurs as hydrogen sulphide or sulphide ions in a pH-dependent equilibrium.

$$H_2S \leftrightarrow HS^- \leftrightarrow S^{2-}$$

Below pH 7, H_2S becomes gradually more important while above pH 9 it is S^{2-}. Between pH 7 and 9 virtually all sulphide is present as HS^-. H_2S is a gas that can enter the cell by passive diffusion. However, cyanobacteria capable of anoxygenic photosynthesis are apparently capable of uptake of the sulphide ion. Sulphide may also react with elemental sulphur to form polysulphides. It was thought that this process could occur only in microbial mats in which the amount of iron is not sufficient to keep the level of free sulphide low (Jørgensen and Cohen 1977). However, Visscher (1992) measured very high concentrations of polysulphides in a cyanobacterial mat, indicating that this compound may be more common than previously assumed. While on the one hand polysulphides are an order of magnitude more toxic for most organisms than sulphide, on the other hand it may serve as the form of elemental sulphur that is transported in cells (Steudel et al. 1990). The microbial mat purple sulphur bacterium *Thiocapsa roseopersicina* is capable of anoxygenic photosynthesis at the expense of polysulphide (Visscher et al. 1990).

4.9 Interactions of Cyanobacteria with Iron

Iron is one of the most abundant elements on Earth and it has several important functions in microbial mats. Iron occurs in three oxidation states: elemental iron Fe^o, ferrous (reduced iron), Fe^{2+} and ferric (oxidized iron), Fe^{3+}. Ferric iron is virtually insoluble and in the presence of oxygen ferrous iron is readily oxidized, except under acidic conditions (pH < 2). Elemental iron is not stable in nature because it will also be oxidized. Thus, in the presence of oxygen at physiological pH iron is hardly available for organisms and aerobic microorganisms often produce compounds that have a high affinity for ferric iron. These siderophores bind iron and transport it into the cells.

Iron is an essential micronutrient for all organisms and also its redox behaviour gives rise to its biological importance. Iron occurs in a number of enzymes that act as electron carriers, such as cytochromes, in respiratory electron transport chains and ferredoxins which serve as electron donors to a variety of processes (including N_2 fixation, nitrate- and sulphate reduction) in the cell, and indirectly, CO_2 fixation. Moreover, iron is an important co-factor in enzymes such as nitrogenase and nitrate reductase. In addition to this assimilatory metabolism of iron, the dissimilatory iron metabolism is of importance in microbial mats and other environments. Ferric iron may serve as electron acceptor in anaerobic respiration (Lovley 1991). Under acid conditions, ferrous iron can be oxidized aerobically by the chemolithotrophic, autotrophic bacterium *Thiobacillus ferrooxidans* (Leduc and Ferroni 1994). Under neutral conditions, ferrous iron is rapidly oxidized by oxygen (Druschel et al. 2008). However, *Gallionella ferruginea* and *Leptothrix ochracea* oxidize iron to support an autotrophic mode of metabolism (Hallbeck and Pedersen 1991; Carlile and Dudeney 2000). Instead of competing with the chemical reaction they rather seem to compete with the autocatalysis of iron oxidation as the result of their own activity (Rentz et al. 2007). Ferrous iron may also serve as electron donor in anoxygenic photosynthesis by specialized purple bacteria (Widdel et al. 1993; Ehrenreich and Widdel 1994). The fourth biologically controlled iron transformation is the formation of magnetite in magnetotactic bacteria and in a variety of other organisms (Stolz 1993).

In microbial mats iron is often present in high amounts (Wieland et al. 2005). In coastal and hypersaline microbial mats suspended iron oxides present in seawater precipitate in the sediment. Iron may precipitate either as oxides and hydroxides, siderite ($FeCO_3$) or as iron sulphide (FeS) and pyrite (FeS_2). Iron readily reacts with sulphide:

$$2Fe^{3+} + H_2S \rightarrow 2Fe^{2+} + S^o + 2H^+$$

$$2Fe^{2+} + 2H_2S \rightarrow 2FeS + 4H^+$$

$$2Fe^{3+} + 3H_2S \rightarrow 2FeS + S^o + 6H^+$$

FeS is virtually insoluble. Thus both ferric and ferrous iron is important in immobilizing the toxic sulphide. Ferrous iron including FeS will react with oxygen both chemically as well as biologically. In microbial mats oxygen supersaturation may present a problem for cyanobacterial growth and the presence of ferrous iron may aid in keeping the partial pressure of oxygen low (Wieland et al. 2005). Moreover, the oxidation of iron in siderite will result in the liberation of CO_2.

In coastal and hypersaline microbial mats a layer of oxidized iron is often observed between the layer of cyanobacteria and the anoxic layers below (Fig. 4.2). When purple sulphur bacteria are present, this layer of oxidized iron usually separates them from the cyanobacteria. The origin of this layer of oxidized iron is not precisely known. Since this layer is generally found at the transition of the oxic-anoxic layers, ferric iron may be produced by chemical oxidation of ferrous iron or biologically by iron-oxidizing bacteria. When oxygen is unavailable due to the continuously migrating oxic-anoxic transition zone, denitrifying bacteria could be responsible for the anaerobic iron oxidation (Straub et al. 1996) although in many microbial mats the amount of nitrate is probably too low to allow for this process. Cohen (1989) supposed that some mat-forming cyanobacteria were capable of iron-dependent anoxygenic photosynthesis. However, it is more likely that the iron is in fact oxidized by oxygen evolved by photosynthesis. Alternatively, anoxygenic phototrophic purple bacteria that use iron as electron donor may have produced the layer of ferric iron. Such organisms have been

isolated from freshwater and marine sediments, including intertidal mud (Widdel et al. 1993; Ehrenreich and Widdel 1994). Also green sulphur bacteria have been shown to use Fe^{2+} as an electron donor in anoxygenic photosynthesis (Crowe et al. 2008). Although such anoxygenic phototrophic bacteria may be responsible for the layer of ferric iron found in microbial mats this has not been demonstrated (Pierson and Parenteau 2000). Moreover, other evidence showed that the oxidation of iron in microbial mats was entirely the result of the oxygen production by the cyanobacteria (Trouwborst et al. 2007). In fact, reduced iron stimulated photosynthesis in cyanobacterial mats which led to higher oxygen levels and higher pH resulting in the precipitation of iron oxides (Pierson et al. 1999).

The formation of distinct layers of oxidized iron in microbial mats may well have resulted in the formation of so-called Banded Iron Formations (BIFs) formed during the Archean and Proterozoic ages. Banded Iron Formations are finely layered sedimentary rocks composed mainly of silica and iron oxides (James and Trendall 1982). BIFs were deposited over large areas and several thousands are known. Although the majority is only few meters thick and covers a limited area, others are several hundreds of meters thick and extend over many thousands of square kilometres (James and Trendall 1982). The iron content of BIFs is typically in the range of 24–35%, which is 5–7 times more than normally found in the crust. These iron formations are therefore of great economic importance. The silica content of BIF's is about 45%. Together, iron oxides and silica may make up to 90% of the weight of BIF. Iron oxides and silica (chert) occur in alternating layers. The cherty banded iron formation of Hamersley Basin, Australia, is one of the largest in the world and is characterized by stratification at different scales. At the millimetre scale microbands of iron minerals are recognized, separated at the centimetre scale by mesobands of chert. Regular banding is seen at the meter scale (macrobands) (James and Trendall 1982). Over 90% of the deposits are from the early Proterozoic age (2,500–1,900 Million years). Although it is tempting to assume a biological basis for the genesis of these cherty iron stromatolites, so far evidence of biogenesis has not emerged. Because of the fact that BIFs were overwhelmingly present during the early Proterozoic this has also been taken as evidence for the oxygenation of the earth's atmosphere which started 2,300 Million years ago. One mechanism for BIF formation may be the chemical oxidation of ferrous iron with oxygen evolved by oxygenic photosynthesis, most likely by cyanobacteria (Trouwborst et al. 2007). The huge amounts of ferrous iron in the earth's crust would act like a buffer and prevent the oxygenation of the atmosphere until most of the iron was oxidized. Although less abundant, the fact that BIF's are also known from the mid Archean ($3.4–2.9 \times 10^9$ years) might indicate other mechanisms. Geological evidence shows that until 2.0×10^9 years ago the oxygen level of the earth's atmosphere was still quite low. High energy solar UV irradiation (200–300 nm range) could freely reach the surface of the earth where it could be absorbed by ferrous iron, resulting in the formation of ferric iron and H_2 which escaped into the atmosphere (Cairns-Smith 1978). This reaction has been experimentally proven to be a possible explanation for the precipitation of ferric iron.

Ferrous and ferric iron strongly absorbs in the region 220–270 nm, UV light that is deleterious for organisms. It has been suggested that both ferrous and ferric iron play an important role in protection from UV irradiation because they provide an effective UV screen (Pierson and Olson 1989). It is assumed that the flux of UV irradiation that reached the earth surface during the early Precambrian was very high since the oxygen-free atmosphere would scarcely attenuate it. It is also known that microbial life developed on earth during this period, particularly in stromatolites. This life was apparently not arrested by the high UV flux. Although UV-C light does not reach the earth surface because it is completely absorbed by the earth's atmosphere, it is interesting that some mat-forming cyanobacteria such as *Microcoleus chthonoplastes* accumulate large amounts of iron at the outer polysaccharide sheath (Stal 1994). Iron is bound to negatively charged polysaccharides, particularly through the presence of uronic acids and precipitates at the sheath (Bender et al. 1994). Acidic extracellular polymeric substances containing carboxyl groups have been shown to mediate iron oxide mineralization (Chan et al. 2009). This has also been seen in a new cyanobacterium ('Chroogloeocystis siderophila') isolated from an iron-depositing hot spring microbial mat (Brown et al. 2005). This strain possesses elevated requirements for iron and also tolerates high levels of iron, making this organism well adapted to thrive in high iron environments. 'C. siderophila' failed to grow at low (8 μM) and at very high (1,000 μM) concentrations of Fe^{3+}. Although this iron is present in an insoluble form, its toxicity at high concentrations may be through the binding of iron precipitates to the outer sheath. Ferric iron is reduced through this EPS or through other cellular processes associated with it, or even under the influence of light. The ferrous iron produced in this way can be taken up by the cell but when in excess it may cause a problem inside the cell or it may draw excessively on the pool of reducing equivalents. Brown et al. (2005) suggested that the accumulation of iron served as a pool of iron for times of low iron availability but this seems unnecessary when thriving in a high iron environment. However, the iron precipitates bound to the outer sheath may present a way for the uptake of iron in organisms that lack siderophores. The sheath EPS would then serve as a siderophore.

There may be other advantages of accumulating iron precipitates by cyanobacteria. One of the possibilities is that it evolved from an ancient UV screen to serve new functions

for the cyanobacteria or for the ecosystem as a whole. For instance, the accumulation of iron by mat-forming cyanobacteria has been shown to protect the organism from sulphide produced either by sulphate-reducing bacteria living in the immediate vicinity of the cyanobacteria or produced by themselves through the reduction of elemental sulphur. Hence, the bound ferric hydroxides may represent a buffer against toxic sulphide, which reacts to produce ferrous iron and iron sulphide. Another possibility is that ferrous iron will react with oxygen, keeping its concentration low and minimizing photorespiration which would lead to a loss of fixed carbon. A low partial pressure of oxygen is essential for the cell in order to minimize the oxygenase reaction of the CO_2-fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) The layer of ferric iron may therefore present an efficient barrier between the aerobic and anaerobic parts of the system (Stal 2001). Finally, *M. chthonoplastes* is also capable of reducing ferric iron probably using it as an electron acceptor during anaerobic dark metabolism (Stal 1994).

Iron oxides form complexes with phosphate which is then immobilized and unavailable as source of phosphate. It is liberated when the iron is reduced. Hence, the cycle of oxidation and reduction of iron may also be important for the temporal binding and storage of phosphate in a microbial mat.

4.10 Phosphorus in Microbial Mats

Few studies have addressed the role of phosphorus in microbial mats. This is remarkable because phosphate is involved in a variety of geochemical reactions that are important in mats and it is indispensible for growth and metabolic activity for all forms of life, including cyanobacteria. The almost complete ignorance of phosphorus in the study of microbial mats is also in strong contrast with the attention it receives in the study of phytoplankton. Generally, nitrogen or phosphorus limits growth of phytoplankton and most likely this applies also to cyanobacteria that form microbial mats.

Typically about 3% dry mass of cells consists of phosphorus, but some cells can store phosphate as polyphosphate, which increases their phosphorus content. Cyanobacteria take up orthophosphate (H_3PO_4) which is the most common form of inorganic phosphorus.

The solubility of orthophosphate is controlled by elements such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+} and Al^{3+}. In seawater, the solubility of orthophosphate is predominantly controlled by Ca^{2+}, which at a suitable pH (7.4–8.1) produces the virtually insoluble hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; solubility product 1.53×10^{-112}) (Ehrlich 1996). In addition, phosphate may also form an insoluble precipitate with ferric iron ($FePO_4 \cdot 2H_2O$, strengite, solubility product 1.35×10^{-18}). Phosphate may be liberated from these insoluble minerals by microbial activity. The mechanisms include:

(i) production of organic acids
(ii) production of chelators
(iii) dissimilatory reduction of ferric iron
(iv) production of sulphide (Ehrlich 1996).

The latter can react with ferric iron phosphate according to:

$$2FePO_4 + 3H_2S \rightarrow 2FeS + 2H_3PO_4 + S^o$$

All these processes are likely to occur in microbial mats, but the phosphate liberated will be taken up immediately by the microbial community. Hence, the occurrence of free orthophosphate ion in microbial mats is expected to be negligible. Any organic phosphates must be cleaved hydrolytically by phosphatases.

As argued in Sect. 4.7, the growth of most cyanobacterial mats in coastal environments seems to be limited by nitrogen. However, microbial mats formed by heterocystous cyanobacteria are more likely to become phosphate-limited because N_2 fixation provides all the nitrogen needed for their growth. Mats built by non-heterocystous cyanobacteria are probably still nitrogen-limited as a result of impairment of N_2 fixation by oxygen. However, as is shown in Table 4.5, phosphate fertilization of such a mat resulted in a dramatic increase of N_2 fixation. Similar observations were made by Camacho and De Wit (2003) for hypersaline microbial mats and by Pinckney et al. (1995) in stromatolitic microbial mats in the Bahamas. While in hypersaline microbial mats the addition of nitrogen resulted in a shift in the community from cyanobacteria to diatoms without increasing the photosynthetic capacity of the mat, phosphate additions greatly stimulated the cyanobacterial community and their capacity of N_2 fixation. Obviously, these mats were co-limited by phosphate and nitrogen. It is known that N_2 fixation requires a certain amount of phosphate for optimal performance (de Nobel et al. 1997). The effect of phosphate fertilization on N_2 fixation may also have been indirect since it also caused a strong decrease in dissolved oxygen in the mat. The latter explanation is supported by the fact that N_2 fixation was also stimulated by other treatments that resulted in a decrease of oxygen (Table 4.5). Phosphate fertilization may also have stimulated heterotrophic bacterial activity and consequently oxygen uptake. The addition of phosphate stimulated gross photosynthesis and oxygen consumption equally well in the hypersaline microbial mat so that net photosynthesis remained unaltered (Ludwig et al. 2006). Hence, the microbial community as a whole was phosphate limited. However, high phosphate (1 mM) additions inhibited photosynthesis but not oxygen consumption. This was possibly due to chemical interactions of phosphate with iron or calcium ions, influencing their availability (Elser et al. 2005). Phosphate limitation caused very high C:P ratios which

constrains the quality of the food for herbivores and therefore minimizes grazing activity (Elser et al. 2005). Phosphate fertilization of coastal mats resulted in a considerable increase of chlorophyll *a* and a shift in cyanobacterial species composition from an *Oscillatoria*-dominated community to one with mainly *Phormidium*-type forms (Stal unpublished).

Although phosphorus may occur in other oxidation states (from +5 to −3), it is not important in redox reactions, as is the case with nitrogen and sulphur. Bacteria readily oxidize any reduced phosphorus, both aerobically and anaerobically. The reduction of orthophosphate is thermodynamically not favorable and is therefore not important for dissimilatory purposes. Hence, the microbial phosphorus cycle consists predominantly of the uptake of inorganic phosphate and the liberation by excretion or autolysis of organic phosphate, which is subsequently mineralized by phosphatases.

Although almost all phosphate on earth is present in the oxidized (+5) form, it has become clear that the more reduced form phosphonate (+3) plays an important role in many organisms (White and Metcalf 2007). Phosphonates are characterized by a very stable C-P bond. The potential of the use of phosphonate as a source of phosphorus has been proposed for the marine planktonic filamentous cyanobacterium *Trichodesmium* (Dyhrman et al. 2006) and the transporter gene *phnD* has been found in marine picocyanobacteria (IIikchyan et al. 2009). The genes that code for the enzymes that are capable of hydrolyzing this bond may have been spread by lateral gene transfer (Huang et al. 2005). In the ocean the source of phosphonates may in fact be the cyanobacterium *Trichodesmium*, of which 10% of the phosphorus is present as phosphonate (Dyhrman et al. 2009). Hot spring microbial mats may constitute up to 5% as phosphonate and may therefore represent an important source of phosphorus, when other sources become unavailable (Adam et al. 2008). The unicellular mat-forming cyanobacterium *Synechococcus* OS-B' possesses genes for the transport of metabolism of phosphonates which were transcribed upon phosphate starvation. This organism could become adapted to growth at the expense of phosphonate even when inorganic phosphate is the dominant source of phosphorus in these mats and phosphonate appeared to be inhibitory in the short term (Adam et al. 2008). The source of phosphonate in microbial mats remains unknown as well as whether it is common in microbial mats.

Phosphate may be stored in mineral deposits such as phosphorite, apatite, strengite, and other forms. Phosphorite deposits are usually found in coastal waters or shallow seas. They can be formed authigenically when soluble phosphate reacts with calcium to form calcium phosphate or by diagenesis when phosphate replaces carbonate in calcareous concretions (Ehrlich 1996). Both processes are probably biologically controlled. The model of Piper and Codespoti (1975) explains phosphorite formation in the marine environment from the mineralization of organic matter below the oxygen minimum layer, where it is coupled to denitrification. This results in excess inorganic phosphate compared to combined nitrogen. Upwelling transports phosphate to the sea surface, where it precipitates with calcium. This model could also apply to microbial mats where the same processes take place. Such phosphorite accumulation has been observed in cyanobacterial mats found on the bottom of small brackish ponds of atolls in French Polynesia called *kopara* (Rougerie et al. 1997). Dahanayake and Krumbein (1985) also reported phosphorite formed by a microbial mat, but concluded that fungi rather than cyanobacteria produced this particular fossil mat.

4.11 Conclusions

Laminated microbial mats are often considered to be recent analogues of fossil Precambrian stromatolites. Stromatolites are laminated lithified structures that have been formed by growth and metabolism of microorganisms. Studies of carbon isotope ratios provide evidence that photosynthesis was involved in the formation of stromatolites and the discovery of microfossils supports the idea that cyanobacteria have built these formations. However, modern microbial mats rarely lithify and doubts have been raised as to whether these systems really can be considered as analogues. Moreover, the sedimentary record may be biased because lithified mats have a greater potential of preservation. Nevertheless, non-lithifying mats have also left their traces in the fossil record and therefore we know that they have existed throughout the geological history. There are a limited number of examples of microbial mats that calcify and form more or less laminated lithified structures which have morphologies very similar to the Precambrian examples. The comparison of lithifying and non-lithifying microbial mats has provided deeper understanding of the factors that determine the processes leading to lithification.

In the majority of examples of microbial mats, cyanobacteria play a key role in their formation. Cyanobacteria are oxygenic phototrophic bacteria and many species are capable of using dinitrogen (N_2) as their only source of nitrogen. Hence, these organisms have a minimum requirement to proliferate, which is important considering the harsh conditions in which microbial mats often develop. Only extreme conditions will limit the biodiversity and exclude higher grazing organisms so that cyanobacteria accumulate to the dense community that produces a mat. Cyanobacteria have a number of additional properties that make them excellent model organisms for forming microbial mats. Many species are motile through gliding movement, which allows them to position themselves under optimal conditions. Light and possibly chemical factors serve as signals to direct the movement of the organisms. Many cyanobacteria are further characterized

by a high affinity for light and reach maximum rate of photosynthesis at very low light intensities and have low compensation points. The requirement for energy for maintenance purposes is low. Cyanobacteria often have high affinities for nutrients and perhaps even more important, they possess storage possibilities for a variety of growth factors. In addition, their metabolic versatility and reactivity are important properties of cyanobacteria. For instance, cyanobacteria are not only photoautotrophs that perform oxygenic photosynthesis but many are also capable of anoxygenic photosynthesis. The majority of mat-forming cyanobacteria is even capable of performing oxygenic and anoxygenic photosynthesis in concert, allowing maximum flexibility and reactivity to quickly changing environmental conditions. Whereas aerobic respiration of endogenous glycogen seems to be the normal metabolism in the dark, this does not usually occur in microbial mats, which often are devoid of oxygen during the night. However, most, if not all, mat forming cyanobacteria are capable of fermentation.

Growth and metabolic activity of the cyanobacteria introduce organic matter in the microbial mat system and its degradation will drive the growth of other micro-organisms in microbial mats. Although some organic matter may become liberated into the environment by death and lysis of the cyanobacteria, this seems not to be most important. In mature microbial mats there is hardly any net growth of cyanobacteria despite the high rates of photosynthesis. Organic matter may become liberated as a result of photorespiration, fermentation, excretion of organic solutes and the secretion of extracellular polymeric substances (EPS), notably polysaccharides. Cyanobacteria may produce a well-defined polysaccharide sheath. This is often a structural component of the cell envelope of cyanobacteria. However, cyanobacteria may also produce vast amounts of mucilage which is not or only partly associated with the organism. Mucilage is often composed of recalcitrant polysaccharides. It produces a matrix in which the microbial mat is embedded. This material is sticky and it will glue sediment particles and organisms together, giving stability to the sediment surface. Since this matrix cannot be mixed it presents a diffusive barrier. The polysaccharide matrix is therefore responsible for the accumulation and supersaturation with oxygen in the light and likewise for the anoxic conditions that occur during the night. Of particular importance is the role that EPS probably plays in calcification. It is likely that EPS inhibits this process and that it serves as an anti-calcification agent. This may be either by preventing growth of small calcite crystallization nuclei or by binding Ca^{2+}. Therefore cyanobacterial mats in which a high amount of mucus is produced will not calcify. The production of mucus in cyanobacteria may be related to unbalanced growth. Unbalanced growth occurs when one growth factor is in shortage. Nitrogen limitation is well known as a factor that stimulates the secretion of mucus. Marine microbial mats are often developing under conditions of nitrogen shortage. This is evidenced by the fact that these mats are built by N_2-fixing cyanobacteria. These mats usually consist of non-heterocystous diazotrophic cyanobacteria that are inefficient in fixing dinitrogen because they lack an effective mechanism to protect nitrogenase from oxygen. This is particularly the case when during the day very high levels of oxygen occur in the mat. At night oxygen is absent and therefore N_2 fixation in these mats occurs predominantly then. However, the limited amount of energy and low-potential electrons that can be generated under such conditions will not allow the fixation of ample dinitrogen. Heterocystous cyanobacteria are optimally equipped for N_2 fixation in the light and will not usually face nitrogen-limited growth. However, as stated above, such cyanobacteria are largely excluded from microbial mats. Possibly heterocystous cyanobacteria cannot tolerate high levels of sulphide or dark anoxic conditions. Also the absence of gliding motility in heterocystous cyanobacteria and the weak connection between the heterocyst and the vegetative cell may exclude these organisms from environments with high rates of sedimentation. It seems reasonable to assume that Precambrian microbial mats did not face nitrogen limitation and thus might have produced much less mucus, calcification therefore not being inhibited. Future research should investigate the nitrogen state of modern calcifying microbial mats and the role of EPS as anti-calcification agents in non-lithifying mats.

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References

Abed RMM, Garcia-Pichel F, Hernández-Mariné M (2002) Polyphasic characterization of benthic, moderately halophilic, moderately thermophilic cyanobacteria with very thin trichomes and the proposal of *Halomicronema excentricum* gen. nov., sp. nov. Arch Microbiol 177:361–370

Adam MM, Gómez-García MR, Grossman AR, Bhaya D (2008) Phosphorus deprivation responses and phosphonate utilization in a thermophilic *Synechococcus* sp. from microbial mats. J Bacteriol 190:8171–8184

Albertano P, Kovacik L (1996) Light and temperature responses of terrestrial sciaphilous strains of *Leptolyngbya* sp. in cross-gradient cultures. Algol Stud 83:17–28

Allen MM, Smith AJ (1969) Nitrogen chlorosis in blue-green algae. Arch Mikrobiol 69:114–120

Allewalt JP, Bateson MM, Revsbech NP, Slack K, Ward DM (2006) Effect of temperature and light on growth of and photosynthesis by *Synechococcus* isolates typical of those predominating in the Octopus Spring microbial mat community of Yellowstone National Park. Appl Environ Microbiol 72:544–550

Anderson KL, Tayne TA, Ward DM (1987) Formation and fate of fermentation products in hot spring cyanobacterial mats. Appl Environ Microbiol 53:2343–2352

Arp G, Reimer A, Reitner J (2001) Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. Science 292:1701–1704

Artus NN, Somerville SC, Somerville CR (1986) The biochemistry and cell biology of photorespiration. CRC Crit Rev Plant Sci 4:121–147

Awramik SM (1984) Ancient stromatolites and microbial mats. In: Cohen Y, Castenholz RW, Halvorson HO (eds) Microbial mats: stromatolites. Alan R. Liss Inc, New York, pp 1–22, 498 pp

Badger MR, Andrews TJ (1982) Photosynthesis and inorganic carbon usage by the marine cyanobacterium, *Synechococcus* sp. Plant Physiol 70:517–523

Bak F, Pfennig N (1987) Chemolithotrophic growth of *Desulfovibrio sulfodismutans* sp. nov. by disproportionation of inorganic sulfur compounds. Arch Microbiol 147:184–189

Bar-Or Y, Shilo M (1987) Characterization of macromolecular flocculants produced by *Phormidium* sp. strain J-1 and by *Anabaenopsis circularis* PCC 6720. Appl Environ Microbiol 53:2226–2230

Bar-Or Y, Shilo M (1988) The role of cell-bound flocculants in coflocculation of benthic cyanobacteria with clay particles. FEMS Microbiol Ecol 53:169–174

Bar-Or Y, Kessel M, Shilo M (1985) Modulation of cell surface hydrophobicity in the benthic cyanobacterium *Phormidium* J-1. Arch Microbiol 142:21–27

Bateson MM, Ward DM (1988) Photoexcretion and fate of glycolate in a hot spring cyanobacterial mat. Appl Environ Microbiol 54:1738–1743

Bauer MR, Haddad RI, Des Marais DJ (1991) Method for determining stable isotope ratios of dissolved organic carbon in interstitial and other natural marine waters. Mar Chem 33:335–351

Bauersachs T, Kremer B, Schouten S, Sinninghe Damsté JS (2009) A biomarker and $\delta^{15}N$ study of thermally altered Silurian cyanobacterial mats. Org Geochem 40:149–157

Bauld J (1984) Microbial mats in marginal marine environments: Shark Bay, Western Australia, and Spencer Gulf, South Australia. In: Cohen Y, Castenholz RW, Halvorson HO (eds) Microbial mats: stromatolites. Alan R. Liss Inc, New York, pp 39–58, 498 pp

Baumgartner LK, Reid RP, Dupraz C, Decho AW, Buckley DH, Spear JR, Przekop KM, Visscher PT (2006) Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sediment Geol 185:131–145

Bebout BM, Garcia-Pichel F (1995) UV B-induced vertical migrations of cyanobacteria in a microbial mat. Appl Environ Microbiol 61:4215–4222

Bender J, Rodriguezeaton S, Ekanemesang UM, Phillips P (1994) Characterization of metal-binding bioflocculants produced by the cyanobacterial component of mixed microbial mats. Appl Environ Microbiol 60:2311–2315

Bergman B, Gallon JR, Rai AN, Stal LJ (1997) N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol Rev 19:139–185

Berner R, Jensen T (1982) Ultrastructure of two hypolithic cyanobacteria from the Negev desert of Israel. Cytobios 35:7–18

Bertocchi C, Navarini L, Cesaro A (1990) Polysaccharides from cyanobacteria. Carbohydr Polym 12:127–153

Bhaya D (2004) Light matters: phototaxis and signal transduction in unicellular cyanobacteria. Mol Microbiol 53:745–754

Bolhuis H, Severin I, Confurius-Guns V, Wollenzien UIA, Stal LJ (2010) Horizontal transfer of the nitrogen fixation gene cluster in the cyanobacterium *Microcoleus chthonoplastes*. ISME J 4:121–130

Borman AH, De Jong EW, Huizinga M, Kok DJ, Westbroek P, Bosch L (1982) The role in $CaCO_3$ crystallization of an acid Ca^{2+}-binding polysaccharide associated with coccliths of *Emiliania huxleyi*. Eur J Biochem 129:179–183

Borman AH, De Jong EW, Thierry R, Westbroek P, Bosch L (1987) Coccolith-associated polysaccharides from cells of *Emiliania huxleyi* (Haptophyceae). J Phycol 23:118–123

Bosak T, Greene SE, Newman DK (2007) A likely role for anoxygenic photosynthetic microbes in the formation of ancient stromatolites. Geobiology 5:119–126

Boudreau BP, Canfield DE (1993) A comparison of closed- and open-system models for porewater pH and calcite-saturation state. Geochim Cosmochim Acta 57:317–334

Braissant O, Decho AW, Przekop KM, Gallagher KL, Glunk C, Dupraz C, Visscher PT (2009) Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat. FEMS Microbiol Ecol 67:293–307

Brock TD (1975) Effect of water potential on a *Microcoleus* (Cyanophyceae) from a desert crust. J Phycol 11:316–320

Brock TD (1978) Thermophilic microorganisms and life at high temperatures. Springer, New York, 465 pp

Brocks JJ, Logan GA, Buick R, Summons RE (1999) Archean molecular fossils and the early rise of eukaryotes. Science 285:1033–1036

Brown II, Mummey D, Cooksey KE (2005) A novel cyanobacterium exhibiting an elevated tolerance for iron. FEMS Microbiol Ecol 52:307–314

Budinoff CR, Hollibaugh JT (2008) Arsenite-dependent photoautotrophy by an *Ectothiorhodospira*-dominated consortium. ISME J 2:340–343

Caiola MG, Ocampo-Friedmann R, Friedmann EI (1993) Cytology of long-term desiccation in the desert cyanobacterium *Chroococcidiopsis* (Chroococcales). Phycologia 32:315–322

Caiola MG, Billi D, Friedmann EI (1996) Effect of desiccation on envelopes of the cyanobacterium *Chroococcidiopsis* sp. (Chroococcales). Eur J Phycol 31:99–105

Cairns-Smith AG (1978) Precambrian solution photochemistry, inverse segregation, and banded iron formations. Nature 276:807–808

Camacho A, de Wit R (2003) Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. Aquat Microb Ecol 32:261–273

Camacho A, Rochera C, Silvestre JJ, Vicente E, Hahn MW (2005) Spatial dominance and inorganic carbon assimilation by conspicuous autotrophic biofilms in a physical and chemical gradient of a cold sulfurous spring: the role of differential ecological strategies. Microb Ecol 50:172–184

Campbell SE (1979) Soil stabilization by a prokaryotic desert crust: implications for Precambrian land biota. Orig Life 9:335–348

Canfield DE, Des Marais DJ (1991) Aerobic sulfate reduction in microbial mats. Science 251:1471–1473

Canfield DE, Des Marais DJ (1994) Cycling of carbon, sulfur, oxygen and nutrients in a microbial mat. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 255–263, 463 pp

Canfield DE, Raiswell R (1991) Carbonate precipitation and dissolution. Its relevance to fossil preservation. In: Allison PA, Briggs DEG (eds) Taphonomy: releasing the data locked in the fossil record. Topics in geobiology, vol 9. Plenum Press, New York, pp 411–453, 546 pp

Canfield DE, Thamdrup B (1996) Fate of elemental sulfur in an intertidal sediment. FEMS Microbiol Ecol 19:95–103

Capone DG, O'Neil JM, Zehr J, Carpenter EJ (1990) Basis for diel variation in nitrogenase activity in the marine planktonic cyanobacterium *Trichodesmium thiebautii*. Appl Environ Microbiol 56:3532–3536

Carlile MJ, Dudeney AWL (2000) A microbial mat composed of iron bacteria. Microbiology 146:2092–2093

Castenholz RW (1973) Movements. In: Carr NG, Whitton BA (eds) The biology of blue-green algae. Blackwell Scientific Publications, Oxford, pp 320–339, 676 pp

Castenholz RW (1976) The effect of sulfide on the blue green algae of hot springs. I. New Zealand and Iceland. J Phycol 12:54–68

Castenholz RW (1977) The effect of sulfide on the blue-green algae of hot springs. II. Yellowstone National Park. Microb Ecol 3:79–105

Castenholz RW (1982) Motility and taxes. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publishers, Oxford, pp 413–439, 688 pp

Castenholz RW, Utkilen HC (1984) Physiology of sulfide tolerance in a thermophilic *Oscillatoria*. Arch Microbiol 138:299–305

Castenholz RW, Jørgensen BB, Damelio E, Bauld J (1991) Photosynthetic and behavioral versatility of the cyanobacterium *Oscillatoria boryana* in a sulfide-rich microbial mat. FEMS Microbiol Ecol 86:43–58

Chafetz HS (1994) Bacterially induced precipitates of calcium carbonate and lithification of microbial mats. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments. BIS Verlag, Oldenburg, pp 149–163, 526 pp

Chafetz HS, Buczynski C (1992) Bacterially induced lithification of microbial mats. Palaios 7:277–293

Chan CS, Fakra SC, Edwards DC, Emerson D, Banfield JF (2009) Iron oxyhydroxide mineralization on microbial extracellular polysaccharides. Geochim Cosmochim Acta 73:3807–3818

Chen M, Schliep M, Willows RD, Cai Z-L, Neilan BA, Scheer H (2010) A red-shifted chlorophyll. Science. doi:10.1126/science.1191127

Choi JS, Chung YH, Moon YJ, Kim C, Watanabe M, Song P-S, Joe C-O, Bogorad L, Park YM (1999) Photomovement of the gliding cyanobacterium *Synechocystis* sp. PCC 6803. Photochem Photobiol 70:95–102

Christensen BE, Kjosbakken J, Smidsrod O (1985) Partial chemical and physical characterization of two extracellular polysaccharides produced by marine periphytic *Pseudomonas* sp. strain NCMB 2021. Appl Environ Microbiol 50:837–845

Cloud PE, Semikhatov MA (1969) Proterozoic stromatolite zonation. Am J Sci 267:1017–1061

Cohen Y (1989) Photosynthesis in cyanobacterial mats and its relation to the sulfur cycle: a model for microbial sulfur interactions. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 22–36, 511 pp

Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407

Cox G, Benson D, Dwarte DM (1981) Ultrastructure of a cave-wall cyanophyte *Gloeocapsa* NS4. Arch Microbiol 130:165–174

Crowe SA, Jones C, Katsev S, Magen C, O'Neill AH, Sturm A, Canfield DE, Haffner GD, Mucci A, Sundby B, Fowle DA (2008) Photoferrotrophs thrive in an Archean ocean analogue. Proc Natl Acad Sci USA 105:15938–15943

Cullen JJ, Neale PJ (1994) Ultraviolet radiation, ozone depletion, and marine photosynthesis. Photosynth Res 39:303–320

Cypionka H, Widdel F, Pfennig N (1985) Survival of sulfate-reducing bacteria after oxygen stress and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiol Ecol 31:39–45

D'Antoni D'Amelio E, Cohen Y, Des Marais DJ (1989) Comparative functional ultrastructure of two hypersaline submerged cyanobacterial mats: Guerrero Negro, Baja California Sur, Mexico, and Solar Lake, Sinai, Egypt. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 97–113, 511 pp

Dahanayake K, Krumbein WE (1985) Ultrastructure of a microbial mat-generated phosphorite. Miner Deposita 20:260–265

Davey A (1983) Effects of abiotic factors on nitrogen fixation by blue-green algae in Antarctica. Polar Biol 2:95–100

Davey MC, Clarke KJ (1992) Fine structure of a terrestrial cyanobacterial mat from Antarctica. J Phycol 28:199–202

Davey A, Marchant HJ (1983) Seasonal variation in nitrogen fixation by *Nostoc commune* Vaucher at the Vesthold Hills, Antarctica. Phycologia 22:377–385

De Nobel WT, Snoep JL, Westerhoff HV, Mur LR (1997) Interaction of nitrogen fixation and phosphorus limitation in *Aphanizomenon flos-aquae* (Cyanophyceae). J Phycol 33:794–799

De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22:151–175

De Philippis R, Margheri MC, Vincenzini M (1996) Fermentation in symbiotic and free-living cyanobacteria. Arch Hydrobiol 83:459–468

De Philippis R, Sili C, Paperi R, Vincenzini M (2001) Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. J Appl Phycol 13:293–299

De Winder B, Matthijs HCP, Mur LR (1989a) The role of water retaining substrata on the photosynthetic response of three drought tolerant phototrophic micro-organisms isolated from a terrestrial habitat. Arch Microbiol 152:458–462

De Winder B, Pluis J, De Reus L, Mur LR (1989b) Characterization of a cyanobacterial, algal crust in the coastal dunes of the Netherlands. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 77–83, 511 pp

De Winder B, Stal LJ, Mur LR (1990) *Crinalium epipsammum* sp. nov.: a filamentous cyanobacterium with trichomes composed of elliptical cells and containing poly-beta-(1,4) glucan (cellulose). J Gen Microbiol 136:1645–1653

De Wit R, Van Gemerden H (1987) Oxidation of sulfide to thiosulfate by *Microcoleus chthonoplastes*. FEMS Microbiol Ecol 45:7–13

De Wit R, Van Gemerden H (1988) Interactions between phototrophic bacteria in sediment ecosystems. Hydrobiol Bull 22:135–145

De Wit R, Van Boekel WHM, Van Gemerden H (1988) Growth of the cyanobacterium *Microcoleus chtonoplastes* on sulfide. FEMS Microbiol Ecol 53:203–209

De Wit R, van den Ende FP, van Gemerden H (1995) Mathematical simulation of the interactions among cyanobacteria, purple sulfur bacteria and chemotrophic sulfur bacteria in microbial mat communities. FEMS Microbiol Ecol 17:117–135

Decho AW (1990) Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. Oceanogr Mar Biol Annu Rev 28:73–153

Decho AW (1994) Molecular-scale events influencing the macroscale cohesiveness of exopolymers. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments. BIS Verlag, Oldenburg, pp 135–148, 526 pp

Decho AW (2000) Microbial biofilms in intertidal systems: an overview. Cont Shelf Res 20:1257–1273

Decho AW, Moriarty DJW (1990) Bacterial exopolymer utilization by a harpacticoid copepod: a methodology and results. Limnol Oceanogr 35:1039–1049

Decho AW, Visscher PT, Reid RP (2005) Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. Palaeogr Palaeoclim Palaeoecol 219:71–86

Défarge C, Trichet J, Couté A (1994a) On the appearance of cyanobacterial calcification in modern stromatolites. Sediment Geol 94:11–19

Défarge C, Trichet J, Maurin A, Hucher M (1994b) Kopara in Polynesian atolls: early stages of formation of calcareous stromatolites. Sediment Geol 89:9–23

Des Marais DJ, Canfield DE (1994) The carbon isotope biogeochemistry of microbial mats. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 289–298, 463 pp

Des Marais DJ, D'Amelio E, Farmer JD, Jørgensen BB, Palmisano AC, Pierson BK (1992) Case study of a modern microbial mat-building community: the submerged cyanobacterial mats of Guerrero Negro, Baja California Sur, Mexico. In: Schopf JW, Klein C (eds) The proterozoic biosphere. A multidisciplinary study. Cambridge University Press, New York, pp 325–333, 1348 pp

Dilling W, Cypionka H (1990) Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiol Lett 71:123–127

Dillon JG, Miller S, Bebout B, Hullar M, Pinel N, Stahl DA (2009) Spatial and temporal variability in a stratified hypersaline microbial mat. FEMS Microbiol Ecol 68:46–58

Donkor V, Häder DP (1991) Effects of solar and ultraviolet radiation on motility, photomovement and pigmentation in filamentous, gliding cyanobacteria. FEMS Microbiol Ecol 86:159–168

Donkor VA, Amewowor DHAK, Häder DP (1993) Effects of tropical solar radiation on the motility of filamentous cyanobacteria. FEMS Microbiol Ecol 12:143–148

Dor I, Paz N (1989) Temporal and spatial distribution of mat microalgae in the experimental solar ponds, Dead Sea area, Israel. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 114–122, 511 pp

Druschel GK, Emerson D, Sutka R, Suchecki P, Luther GW III (2008) Low-oxygen and chemical kinetic constraints on the geochemical niche of neutrophilic iron(II) oxidizing microorganisms. Geochim Cosmochim Acta 72:3358–3370

Dubinin AV, Gerasimenko LM, Zavarzin GA (1992) Nitrogen fixation by cyanobacterium *Microcoleus chthonoplastes* from hypersaline lagoons of lake Sivash. Microbiology 61:593–597

Dupraz C, Visscher PT (2005) Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol 13:429–438

Dupraz C, Visscher PT, Baumgartner LK, Reid RP (2004) Microbe-mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas). Sedimentology 51:745–765

Dupraz C, Reid RP, Braissant O, Decho AW, Norman RS, Visscher PT (2009) Processes of carbonate precipitation in modern microbial mats. Earth Sci Rev 96:141–162

Dyhrman ST, Chappell PD, Haley ST, Moffett JW, Orchard ED, Waterbury JB, Webb EA (2006) Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature 439:68–71

Dyhrman ST, Benitez-Nelson CR, Orchard ED, Haley ST, Pellechia PJ (2009) A microbial source of phosphonates in oligotrophic marine systems. Nat Geosci 2:696–699

Ehrenberg CG (1838) Über das im Jahre 1686 in Curland vom Himmel gefallene Meteorpapier und über dessen Zusammensetzung aus Conferven und Infusorien. Abh Kgl Akad Wiss Berlin, pp 45–58

Ehrenreich A, Widdel F (1994) Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. Appl Environ Microbiol 60:4517–4526

Ehrlich HL (1996) Geomicrobiology. Marcel Dekker Inc, Basel, 719 pp

Eisenhut M, Ruth W, Haimovich M, Bauwe H, Kaplan A, Hagemann M (2008) The photorespiratory glycolate metabolism is essential for cyanobacteria and might have been conveyed endosymbiontically to plants. Proc Natl Acad Sci USA 105:17199–17204

Eloff JN, Steinitz Y, Shilo M (1976) Photooxidation of cyanobacteria in natural conditions. Appl Environ Microbiol 31:119–126

Elser JJ, Schampel JH, Garcia-Pichel F, Wade BD, Souza V, Eguiarte L, Escalante A, Farmer JD (2005) Effects of phosphorus enrichment and grazing snails on modern stromatolitic microbial communities. Freshw Biol 50:1808–1825

Emerson D, Revsbech NP (1994a) Investigation of an iron-oxidizing microbial mat community located near Aarhus, Denmark: field studies. Appl Environ Microbiol 60:4022–4031

Emerson D, Revsbech NP (1994b) Investigation of an iron-oxidizing microbial mat community located near Aarhus, Denmark: laboratory studies. Appl Environ Microbiol 60:4032–4038

Falcón LI, Cerritos R, Eguiarte LE, Souza V (2007) Nitrogen fixation in microbial mat and stromatolite communities from Cuatro Cienegas, Mexico. Microb Ecol 54:363–373

Fechner R (1915) Die Chemotaxis der Oscillarien und ihre Bewegungserscheinungen überhaupt. Z Bot 7:289–364

Ferris MJ, Ruff Roberts AL, Kopczynski ED, Bateson MM, Ward DM (1996) Enrichment culture and microscopy conceal diverse thermophilic *Synechococcus* populations in a single hot spring microbial mat habitat. Appl Environ Microbiol 62:1045–1050

Flores E, Herrero A (1994) Assimilatory nitrogen metabolism and its regulation. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp 487–517, 881 pp

Fouke BW, Farmer JD, Des Marais DJ, Pratt L, Sturchio NC, Burns PC, Discipulo MK (2000) Depositional facies and aqueous-solid geochemistry of travertine-depositing hot springs (Angel Terrace, Mammoth Hot Springs, Yellowstone National Park, USA). J Sediment Res 70:565–585

Friedman I, Lipkin Y, Ocampo-Paus R (1967) Desert algae of the Negev (Israel). Phycologia 6:185–200

Friedrich M, Schink B (1993) Hydrogen formation from glycolate driven by reversed electron transport in membrane vesicles of a syntrophic glycolate-oxidizing bacterium. Eur J Biochem 217: 233–240

Friedrich M, Schink B (1995) Isolation and characterization of a desulforubidin-containing sulfate-reducing bacterium growing with glycolate. Arch Microbiol 164:271–279

Fründ C, Cohen Y (1992) Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl Environ Microbiol 58:70–77

Gallon JR, Hashem MA, Chaplin AE (1991) Nitrogen fixation by *Oscillatoria* Spp under autotrophic and photoheterotrophic conditions. J Gen Microbiol 137:31–39

Garcia-Pichel F, Bebout BM (1996) Penetration of ultraviolet radiation into shallow water sediments: high exposure for photosynthetic communities. Mar Ecol Prog Ser 131:257–262

Garcia-Pichel F, Castenholz RW (1991) Characterization and biological implications od scytonemin, a cyanobacterial sheath pigment. J Phycol 27:395–409

Garcia-Pichel F, Castenholz RW (1994) On the significance of solar ultraviolet radiation for the ecology of microbial mats. In: Stal LJ, Caumette P (eds) Microbial mats. Structure development and environmental significance. Springer, Heidelberg, pp 77–84, 463 pp

Garcia-Pichel F, Pringault O (2001) Cyanobacteria track water in desert soils. Nature 413:380–381

Garcia-Pichel F, Wojciechowski MF (2009) The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PLoS One 4:e7801

Garcia-Pichel F, Mechling M, Castenholz RW (1994) Diel migrations of microorganisms within a benthic, hypersaline mat community. Appl Environ Microbiol 60:1500–1511

Garcia-Pichel F, Prufert-Bebout L, Muyzer G (1996) Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. Appl Environ Microbiol 62:3284–3291

Garlick S, Oren A, Padan E (1977) Occurence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. J Bacteriol 129:623–629

Gebelein CD (1976) Open marine subtidal and intertidal stromatolites (Florida, The Bahamas and Bermuda). In: Walter MR (ed) Stromatolites. Elsevier, Amsterdam, pp 381–388, 790 pp

Gerbaud A, Andre M (1987) An evaluation of the recycling in measurements of photorespiration. Plant Physiol 83:933–937

Gingras M, Hagadorn JW, Seilacher A, Lalonde SV, Pecoits E, Petrash D, Konhauser KO (2011) Possible evolution of mobile animals in association with microbial mats. Nat Geosci. doi:10.1038/NGO1142

Golubić S (1973) The relationship between blue-green algae and carbonate deposits. In: Carr NG, Whitton BA (eds) The biology of blue-green algae. Blackwell Scientific Publications/Univeristy of California Press, Oxford/Berkeley, pp 434–473, 676 pp

Griffin BM, Schott J, Schink B (2007) Nitrite, an electron donor for anoxygenic photosynthesis. Science 316:1870

Griffiths MSH, Gallon JR, Chaplin AE (1987) The diurnal pattern of dinitrogen fixation by cyanobacteria in situ. New Phytol 107:649–657

Guerrero R, Mas J (1989) Multilayered microbial communities in aquatic ecosystems: growth and loss factors. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 37–51, 511 pp

Häder D-P (1987a) Photomovement. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 325–345, 534 pp

Häder D-P (1987b) Photosensory behavior in procaryotes. Microbiol Rev 51:1–21

Häder D-P (1988) Signal perception and amplification in photoresponses of cyanobacteria. Biophys Chem 29:155–159

Häder DP, Kumar HD, Smith RC, Worrest RC (1998) Effects on aquatic ecosystems. J Photochem Photobiol B 46:53–68

Halfen LN, Castenholz RW (1971) Gliding motility in the blue-green alga *Oscillatoria princeps*. J Phycol 7:133–145

Hallbeck L, Pedersen K (1991) Autotrophic and mixotrophic growth *of Gallionella ferruginea*. J Gen Microbiol 137:2657–2661

Hedges SB, Chen H, Kumar S, Wang DY-C, Thompsom AS, Watanabe H (2001) A genomic timescale for the origin of eukaryotes. BMC Evol Biol 1:4

Heijthuijsen JHFG, Hansen TA (1989) Betaine fermentation and oxidation by marine *Desulfuromonas* strains. Appl Environ Microbiol 55:965–969

Heyer H, Stal LJ, Krumbein WE (1989) Simultaneous heterolactic and acetate fermentation in the marine cyanobacterium *Oscillatoria limosa* incubated anaerobically in the dark. Arch Microbiol 151:558–564

Hoehler TM, Albert DB, Alperin MJ, Martens CS (1999) Acetogenesis from CO_2 in an anoxic marine sediment. Limnol Oceanogr 44:662–667

Hoehler TM, Bebout BM, Des Marais DJ (2001) The role of microbial mats in the production of reduced gases on the early Earth. Nature 412:324–327

Hoehler TM, Albert DB, Alperin MJ, Bebout BM, Martens CS, Des Marais DJ (2002) Comparative ecology of H_2 cycling in sedimentary and phototrophic ecosystems. Antonie Van Leeuwenhoek 81:575–585

Hoiczyk E (2000) Gliding motility in cyanobacteria: observations and possible explanations. Arch Microbiol 174:11–17

Howard JB, Rees DC (1994) Nitrogenase: a nucleotide-dependent molecular switch. Annu Rev Biochem 63:235–264

Huang J, Su Z, Xu Y (2005) The evolution of microbial phosphonate degradative pathways. J Mol Evol 61:682–690

Husic DW, Husic HD, Tolbert NE (1987) The oxidative photosynthetic carbon cycle or C_2 cycle. CRC Crit Rev Plant Sci 5:45–100

Ilikchyan IN, Mckay RML, Zehr JP, Dyhrman ST, Bullerjahn GS (2009) Detection and expression of the phosphonate transporter gene *phnD* in marine and freshwater picocyanobacteria. Environ Microbiol 11:1314–1324

Jaeschke A, Op den Camp HJM, Harhangi H, Klimiuk A, Hopmans EC, Jetten MSM, Schouten S, Sinninghe Damsté JS (2009) 16S rRNA gene and lipid biomarker evidence for anaerobic ammonium-oxidizing bacteria (anammox) in California and Nevada hot springs. FEMS Microbiol Ecol 67:343–350

James HL, Trendall AF (1982) Banded iron-formation: distribution in time and paleoenvironmental significance. In: Holland HD, Schidlowski M (eds) Mineral deposits and the evolution of the biosphere. Springer, Heidelberg, pp 199–217, 333 pp

Janson S, Matveyev A, Bergman B (1998) The presence and expression of *hetR* in the non-heterocystous cyanobacterium *Symploca* PCC 8002. FEMS Microbiol Lett 168:173–179

Jones K, Stewart WDP (1969) Nitrogen turnover in marine and brackish habitats. III. The production of extracellular nitrogen by Calothrix scopulorum. J Mar Biol Assoc UK 49:475–488

Jonkers HM, Ludwig R, de Wit R, Pringault O, Muyzer G, Niemann H, Finke N, de Beer D (2003) Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: 'La Salada de Chiprana' (NE Spain). FEMS Microbiol Ecol 44:175–189

Jørgensen BB, Cohen Y (1977) Solar Lake (Sinai) .5. The sulfur cycle of the benthic cyanobacterial mats. Limnol Oceanogr 22:657–666

Jørgensen BB, Des Marais DJ (1988) Optical properties of benthic photosynthetic communities: fiber-optic studies of cyanobacterial mats. Limnol Oceanogr 33:99–113

Jørgensen BB, Nelson DC (1988) Bacterial zonation, photosynthesis, and spectral light distribution in hot spring microbial mats of Iceland. Microb Ecol 16:133–147

Jørgensen BB, Revsbech NP, Blackburn TH, Cohen Y (1979) Diurnal cycle of oxygen and sulfide microgradients and microbial photosynthesis in a cyanobacterial mat sediment. Appl Environ Microbiol 38:46–58

Jørgensen BB, Revsbech NP, Cohen Y (1983) Photosynthesis and structure of benthic microbial mats: micro-electrode and SEM studies of four cyanobacterial communities. Limnol Oceanogr 28:1075–1093

Jørgensen BB, Cohen Y, Revsbech NP (1986) Transition from anoxygenic to oxygenic photosynthesis in *a Microcoleus chthonoplastes* cyanobacterial mat. Appl Environ Microbiol 51:408–417

Jørgensen BB, Cohen Y, Des Marais DJ (1987) Photosynthetic action spectra and adaptation to spectral light distribution in a benthic cyanobacterial mat. Appl Environ Microbiol 53:879–886

Joye SB, Paerl HW (1994) Nitrogen cycling in microbial mats: rates and patterns of denitrification and nitrogen fixation. Mar Biol 119:285–295

Kalkowsky E (1908) Oolith und Stromatolith im Norddeutschen Buntsandstein. Z Dtsch Geol Ges 60:68–125

Kaplan A, Schwarz R, Lieman-Hurwitz J, Ronen-Tarazi M, Reinhold L (1994) Physiological and molecular studies on the response of cyanobacteria to changes in the ambient inorganic carbon concentration. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp 469–485, 881 pp

Karsten U (1996) Growth and organic osmolytes of geographically different isolates of *Microcoleus chthonoplastes* (cyanobacteria) from benthic microbial mats: response to salinity change. J Phycol 32:501–506

Kazmierczak J, Coleman ML, Gruszczynski M, Kempe S (1996) Cyanobacterial key to the genesis of micritic and peloidal limestones in ancient seas. Acta Palaeontol Pol 41:319–338

Kempe S, Kazmierczak J (1990a) Chemistry and stromatolites of the sea-linked Satonda Crater Lake, Indonesia: a recent model for the Precambrian sea? Chem Geol 81:299–310

Kempe S, Kazmierczak J (1990b) Calcium carbonate supersaturation and the formation of in situ calcified stromatolites. In: Ittekkot V, Kempe S, Michaelis W, Spitzy A (eds) Facets of modern biogeochemistry. Springer, Heidelberg, pp 255–278, 433 pp

Kempe S, Kazmierczak J (1993) Satonda crater lake, Indonesia. Hydrogeochemistry and biocarbonates. Facies 28:1–32

Kempe S, Kazmierczak J, Landmann G, Konuk T, Reimer A, Lipp A (1991) Largest known microbialites discovered in Lake Van, Turkey. Nature 349:605–608

Kennard JM, James NP (1986) Thrombolites and stromatolites: two distinct types of microbial structures. Palaios 1:492–503

Kenyon CN, Rippka R, Stanier RY (1972) Fatty acid composition and physiological properties of some filamentous blue-green algae. Arch Microbiol 83:216–236

Kidron GJ, Yaalon DH, Vonshak A (1999) Two causes for runoff initiation on microbiotic crusts: hydrophobicity and pore clogging. Soil Sci 164:18–27

Kiene RP, Visscher PT (1987) Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments. Appl Environ Microbiol 53:2426–2434

Kremer B, Kazmierczak J, Stal LJ (2008) Calcium carbonate precipitation in cyanobacterial mats from sandy tidal flats of the North Sea. Geobiology 6:46–56

Krumbein WE (1974) On the precipitation of aragonite on the surface of marine bacteria. Naturwissenschaften 61:167

Krumbein WE (1979) Photolithotropic and chemoorganotrophic activity of bacteria and algae as related to beachrock formation and degradation (Gulf of Aqaba, Sinai). Geomicrobiol J 1:139–203

Krumbein WE (1983) Stromatolites.The challenge of a term in space and time. Precambrian Res 20:493–531

Krumbein WE, Cohen Y (1977) Primary production, mat formation and lithification: contribution of oxygenic and facultative anoxygenic cyanobacteria. In: Flügel E (ed) Fossil algae. Springer, Berlin, pp 38–56, 375 pp

Krumbein WE, Giele C (1979) Calcification in a coccoid cyanobacterium accociated with the formation of desert stromatolites. Sedimentology 26:593–604

Krumbein WE, Jens K (1981) Biogenic rock varnishes of the Negev Desert (Israel) an ecological study of iron and manganese transformation by cyanobacteria and fungi. Oecologia 50:25–38

Krumbein WE, Cohen Y, Shilo M (1977) Solar Lake (Sinai).4. Stromatolitic cyanobacterial mats. Limnol Oceanogr 22:635–656

Kühl M, Jørgensen BB (1992) Spectral light measurements in microbenthic phototrophic communities with a fiber-optic microprobe coupled to a sensitive diode array detector. Limnol Oceanogr 37:1813–1823

Kulp TR, Hoeft SE, Asao M, Madigan MT, Hollibaugh JT, Fisher JC, Stolz JF, Culbertson CW, Miller LG, Oremland RS (2008) Arsenic(III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California. Science 321:967–970

Lange W (1976) Speculations on a possible essential function of the gelatinous sheath of blue-green algae. Can J Microbiol 22:1181–1185

Lassen C, Ploug H, Jørgensen BB (1992a) Microalgal photosynthesis and spectral scalar irradiance in coastal marine sediments of Limfjorden, Denmark. Limnol Oceanogr 37:760–772

Lassen C, Ploug H, Jorgensen BB (1992b) A fibre-optic scalar irradiance microsensor – application for spectral light measurements in sediments. FEMS Microbiol Ecol 86:247–254

Leduc LG, Ferroni GD (1994) The chemolithotrophic bacterium *Thiobacillus ferrooxidans*. FEMS Microbiol Rev 14:103–119

Lee RY, Joye SB (2006) Seasonal patterns of nitrogen fixation and denitrification in oceanic mangrove habitats. Mar Ecol Prog Ser 307:127–141

Lehmann M, Wöber G (1976) Accumulation, mobilization and turnover of glycogen in the blue-green bacterium *Anacystis nidulans*. Arch Microbiol 111:93–97

Ley RE, Harris JK, Wilcox J, Spear JR, Miller SR, Bebout BM, Maresca JA, Bryant DA, Sogin ML, Pace NR (2006) Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. Appl Environ Microbiol 72:3685–3695

Li P, Liu Z, Xu R (2001) Chemical characterization of the released polysaccharide from the cyanobacterium *Aphanothece halophytica* GR02. J Appl Phycol 13:71–77

Logan BW (1961) Cryptozoon and associate stromatolites from the recent of Shark Bay, Western Australia. J Geol 69:517–533

Lorenz MG, Wackernagel W (1990) Natural genetic transformation of *Pseudomonas stutzeri* by sand-adsorbed DNA. Arch Microbiol 154:380–385

Lorenz MG, Wackernagel W (1994) Bacterial gene transfer by natural genetic transformation in the environment. Microbiol Rev 58:563–602

Lorimer GH (1981) The carboxylation and oxygenation of ribulose 1,5-biphosphate: the primary events in photosynthesis and photorespiration. Annu Rev Plant Physiol 32:349–383

Lorimer GH, Andrews TJ, Tolbert NE (1973) Ribulose diphosphate oxygenase.II. Further proof of reaction products and mechanisms of action. Biochemistry 12:18–23

Lovley DR (1991) Dissimilatory Fe(III) and Mn(IV) reduction. Microbiol Rev 55:259–287

Lowe DR (1980) Stromatolites 3,400-Myr old from the Archean of Western Australia. Nature 284:441–443

Lu W-D, Chi Z-M, Su C-D (2006) Identification of glycine betaine as compatible solute in *Synechococcus* sp. WH8102 and characterization of its *N*-methyltransferase genes involved in betaine synthesis. Arch Microbiol 186:495–506

Ludwig R, Al-Horani FA, de Beer D, Jonkers HM (2005) Photosynthesis-controlled calcification in a hypersaline microbial mat. Limnol Oceanogr 50:1836–1843

Ludwig R, Pringault O, de Wit R, de Beer D, Jonkers HM (2006) Limitation of oxygenic photosynthesis and oxygen consumption by phosphate and organic nitrogen in a hypersaline microbial mat: a microsensor study. FEMS Microbiol Ecol 57:9–17

Mackay MA, Norton RS, Borowitzka LJ (1984) Organic osmoregulatory solutes in cyanobacteria. J Gen Microbiol 130:2177–2191

Mackerras AH, Youens BN, Weir RC, Smith GD (1990a) Is cyanophycin involved in the integration of nitrogen and carbon metabolism in the cyanobacteria *Anabaena cylindrica* and *Gloeothece* grown on light/dark cycles? J Gen Microbiol 136:2049–2056

Mackerras AH, De Chazal NM, Smith GD (1990b) Transient accumulations of cyanophycin in *Anabaena cylindrica* and *Synechocystis* 6308. J Gen Microbiol 136:2057–2065

Malin G, Pearson HW (1988) Aerobic nitrogen fixation in aggregate-forming cultures of the nonheterocystous cyanobacterium *Microcoleus chthonoplastes*. J Gen Microbiol 134:1755–1763

Malin G, Walsby AE (1985) Chemotaxis of a cyanobacterium on concentration gradients of carbondioxide, bicarbonate and oxygen. J Gen Microbiol 131:2643–2652

Margheri MC, Allotta G (1993) Homoacetic fermentation in the cyanobacterium *Nostoc* sp. strain Cc from *Cycas circinalis*. FEMS Microbiol Lett 111:213–217

Marschall C, Frenzel P, Cypionka H (1993) Influence of oxygen on sulfate reduction and growth of sulfate-reducing bacteria. Arch Microbiol 159:168–173

Martin JP (1971) Decomposition and binding action of polysaccharides in soil. Soil Biol Biochem 3:33–34

Martínez-Alonso M, Mir J, Caumette P, Gaju N, Guerrero R, Esteve I (2004) Distribution of phototrophic populations and primary production in a microbial mat from the Ebro Delta, Spain. Int Microbiol 7:19–25

Maryan PS, Eady RR, Chaplin AE, Gallon JR (1986) Nitrogen fixation by *Gloeothece* sp. PCC 6909: respiration and not photosynthesis supports nitrogenase activity in the light. J Gen Microbiol 132:789–796

Mason TR, Von Brunn V (1977) 3-Gyr-old stromatolites from South Africa. Nature 266:47–49

Mazor G, Kidron GJ, Vonshak A, Abeliovich A (1996) The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts. FEMS Microbiol Ecol 21:121–130

McCarren J, Heuser J, Roth R, Yamada N, Martone M, Brahamsha B (2005) Inactivation of *swmA* results in the loss of an outer cell layer in a swimming *Synechococcus* strain. J Bacteriol 187:224–230

McKay RML, Gibbs SP, Espie GS (1992) Effect of dissolved inorganic carbon on the expression of carboxysomes, localization of RubisCO and the mode of inorganic carbon transport in cells of the cyanobacterium *Synechocystis* UTEX 625. Arch Microbiol 159:21–29

Milliman JD (1993) Production and accumulation of calcium carbonate in the ocean: budget of a nonsteady state. Glob Biogeochem Cycle 7:927–957

Minz D, Fishbain S, Green SJ, Muyzer G, Cohen Y, Rittman BE, Stahl DA (1999) Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. Appl Environ Microbiol 65:4659–4665

Miziorko HM, Lorimer GH (1983) Ribulose-1,5-biphosphate carboxylase-oxygenase. Annu Rev Biochem 52:507–535

Moezelaar R, Bijvank SM, Stal LJ (1996) Fermentation and sulfur reduction in the mat-building cyanobacterium *Microcoleus chthonoplastes*. Appl Environ Microbiol 62:1752–1758

Monty CLV (1976) The origin and development of cryptalgal fabrics. In: Walter MR (ed) Stromatolites. Elsevier, Amsterdam, pp 139–249, 790 pp

Moore LR, Post AF, Rocap G, Chisholm SW (2002) Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. Limnol Oceanogr 47:989–996

Mullineaux PM, Gallon JR, Chaplin AE (1981) Acetylene reduction (nitrogen fixation) by cyanobacteria grown under alternating light-dark cycles. FEMS Microbiol Lett 10:245–247

Needoba JA, Foster RA, Sakamoto C, Zehr JP, Johnson KS (2007) Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean. Limnol Oceanogr 52:1317–1327

Neu TR (1992) Microbial "footprints" and the general ability of microorganisms to label interfaces. Can J Microbiol 38:1005–1008

Ng W-O, Grossman AR, Bhaya D (2003) Multiple light inputs control phototaxis in *Synechocystis* sp. starin PCC6803. J Bacteriol 185:1599–1607

Nicholson JAM, Stolz JF, Pierson BK (1987) Structure of a microbial mat at Great Sippewissett Marsh, Cape Cod, Massachusetts. FEMS Microbiol Ecol 45:343–364

Nilsson M, Rasmussen U, Bergman B (2006) Cyanobacterial chemotaxis to extracts of host and nonhost plants. FEMS Microbiol Ecol 55:382–390

Noffke N (2009) The criteria for the biogeneicity of microbially induced sedimentary structures (MISS) in Archean and younger, sandy deposits. Earth Sci Rev 96:173–180

Noffke N, Beukes N, Gutzmer J, Hazen R (2006) Spatial and temporal distribution of microbially induced sedimentary structures: a case study from siliciclastic storm deposits of the 2.9 Ga Witwatersrand Supergroup, South Africa. Precambrian Res 146:35–44

Nold SC, Ward DM (1996) Photosynthate partitioning and fermentation in hot spring microbial mat communities. Appl Environ Microbiol 62:4598–4607

Olson JM (2006) Photosynthesis in the Archean Era. Photosynth Res 88:109–117

Olson JB, Litaker RW, Paerl HW (1999) Ubiquity of heterotrophic diazotrophs in marine microbial mats. Aquat Microb Ecol 19:29–36

Omoregie EO, Crumbliss LL, Bebout BM, Zehr JP (2004) Determination of nitrogen-fixing phylotypes in *Lyngbya* sp. and *Microcoleus chthonoplastes* cyanobacterial mats from Guerrero Negro, Baja California, Mexico. Appl Environ Microbiol 70:2119–2128

Oren A (1988) The microbial ecology of the Dead Sea. Adv Microb Ecol 10:193–229

Oren A, Shilo M (1979) Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica*: sulfur respiration and lactate fermentation. Arch Microbiol 122:77–84

Oren A, Padan E, Avron M (1977) Quantum yields for oxygenic and anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. Proc Natl Acad Sci USA 74:2152–2156

Orpen JL, Wilson JF (1981) Stromatolites at ca. 3,500 Myr and a greenstone graniteunconformity in the Zimbabwean Archaean. Nature 291:218–220

Ortega-Calvo JJ, Stal LJ (1991) Diazotrophic growth of the unicellular cyanobacterium *Gloeothece* sp PCC 6909 in continuous culture. J Gen Microbiol 137:1789–1797

Ortega-Calvo JJ, Stal LJ (1994) Sulphate-limited growth in the N_2-fixing unicellular cyanobacterium *Gloeothece* (Nageli) sp PCC 6909. New Phytol 128:273–281

Ortega-Morales BO, Santiago-Garcia JL, Chan-Bacab MJ, Moppert X, Miranda-Tello E, Fardeau ML, Carrero JC, Bartolo-Perez P, Valadez-Gonzalez A, Guezennec J (2006) Characterization of extracellular polymers synthesized by tropical intertidal biofilm bacteria. J Appl Microbiol 102:254–264

Padan E, Cohen Y (1982) Anoxygenic photosynthesis. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 215–235, 688 pp

Paerl HW, Prufert LE (1987) Oxygen-poor microzones as potential sites of microbial N_2 fixation in nitrogen-depleted aerobic marine waters. Appl Environ Microbiol 53:1078–1087

Paerl HW, Bebout BM, Prufert LE (1989) Naturally occurring patterns of oxygenic photosynthesis and N_2 fixation in a marine microbial mat: physiological and ecological ramifications. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 326–341, 511 pp

Paerl HW, Prufert LE, Ambrose WW (1991) Contemporaneous N_2 fixation and oxygenic photosynthesis in the nonheterocystous mat-forming cyanobacterium *Lyngbya aestuarii*. Appl Environ Microbiol 57:3086–3092

Paerl HW, Pinckney JL, Kucera SA (1995) Clarification of the structural and functional roles of heterocysts and anoxic microzones in the control of pelagic nitrogen fixation. Limnol Oceanogr 40:634–638

Paerl HW, Fitzpatrick M, Bebout BM (1996) Seasonal nitrogen fixation dynamics in a marine microbial mat: potential roles of cyanobacteria and microheterotrophs. Limnol Oceanogr 41:419–427

Palinska KA, Liesack W, Rhiel E, Krumbein WE (1996) Phenotype variability of identical genotypes: the need for a combined approach in cyanobacterial taxonomy demonstrated on *Merismopedia*-like isolates. Arch Microbiol 166:224–233

Papineau D, Walker JJ, Mojzsis SJ, Pace NR (2005) Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. Appl Environ Microbiol 71:4822–4832

Paterson DM (1989) Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behaviour of epipelic diatoms. Limnol Oceanogr 34:223–234

Pearson HW, Howsley R, Kjeldsen CK, Walsby AE (1979) Aerobic nitrogenase activity associated with a non-heterocystous filamentous cyanobacterium. FEMS Microbiol Lett 5:163–169

Pentecost A (1984) Effects of sedimentation and light intensity on mat-forming Oscillatoriaceae with particular reference to *Microcoleus lyngbyaceus* Gomont. J Gen Microbiol 130:983–990

Pentecost A (1988) Growth and calcification of the cyanobacterium *Homoeothrix crustacea*. J Gen Microbiol 134:2665–2671

Pentecost A, Bauld J (1988) Nucleation of calcite on the sheaths of cyanobacteria using a simple diffusion cell. Geomicrobiol J 6:129–135

Pereira S, Zille A, Micheletti E, Moradas-Ferreira P, De Philippis R, Tamagnini P (2009) Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev 33:917–941

Peschek GA (1978) Reduced sulfur and nitrogen compounds and molecular hydrogen as electron donors for anaerobic CO_2 photoreduction in *Anacystis nidulans*. Arch Microbiol 119:313–322

Pierce J (1988) Prospects for manipulating the substrate specificity of ribulose bisphosphate carboxylase/oxygenase. Physiol Plant 72:690–698

Pierson BK (1992) Introduction. In: Schopf JW, Klein C (eds) The proterozoic biosphere. A multidisciplinary study. Cambridge University Press, New York, pp 247–251, 1348 pp

Pierson BK, Olson JM (1989) Evolution of photosynthesis in anoxygenic photosynthetic procaryotes. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 402–427, 511 pp

Pierson BK, Parenteau MN (2000) Phototrophs in high iron microbial mats: microstructure of mats in iron-depositing hot springs. FEMS Microbiol Ecol 32:181–196

Pierson B, Oesterle A, Murphy GL (1987) Pigments, light penetration, and photosynthetic activity in the multi-layered microbial mats of Great Sippewisset salt marsh, Massachusetts. FEMS Microbiol Ecol 45:365–376

Pierson BK, Sands VM, Frederick JL (1990) Spectral irradiance and distribution of pigments in a highly layered marine microbial mat. Appl Environ Microbiol 56:2327–2340

Pierson BK, Parenteau MN, Griffin BM (1999) Phototrophs in high-iron-concentration microbial mats: physiological ecology of phototrophs in an iron-depositing hot spring. Appl Environ Microbiol 65:5474–5483

Pinckney J, Paerl HW, Reid RP, Bebout B (1995) Ecophysiology of stromatolitic microbial mats, Stocking Island, Exuma Cays, Bahamas. Microb Ecol 29:19–37

Piper DZ, Codespoti LA (1975) Marine phosphorite deposits and the nitrogen cycle. Science 179:564–565

Ploug H, Lassen C, Jørgensen BB (1993) Action spectra of microalgal photosynthesis and depth distribution of spectral scalar irradiance in a coastal marine sediment of Limfjorden, Denmark. FEMS Microbiol Ecol 102:261–270

Porubsky WP, Weston NB, Joye SB (2009) Benthic metabolism and the fate of dissolved inorganic nitrogen in intertidal sediments. Estuar Coast Shelf Sci 83:392–402

Potts M (1979) Nitrogen fixation (acetylene reduction) associated with communities of heterocystous and non-heterocystous blue-green algae in mangrove forests of Sinai. Oecologia 39:359–373

Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755–805

Potts M, Friedman EI (1981) Effects of water stress on crypto-endolithic cyanobacteria from hot dessert rocks. Arch Microbiol 130:267–271

Potts M, Ocampo-Friedmann R, Bowman MA, Tozun B (1983) *Chroococcus* S24 and *Chroococcus* N41 (cyanobacteria): morphological, biochemical and genetic characterization and effects of water stress on ultra structure. Arch Microbiol 135:81–90

Pringault O, Garcia-Pichel F (2004) Hydrotaxis of cyanobacteria in desert crusts. Microb Ecol 47:366–373

Rabenstein A, Rethmeier J, Fischer U (1995) Sulphite as intermediate sulphur compound in anaerobic sulphide oxidation to thiosulphate by marine cyanobacteria. Z Naturforsch C 50:769–774

Ramsing NB, Prufert-Bebout L (1994) Motility of *Microcoleus chthonoplastes* subjected to different light intensities quantified by digital image analysis. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 183–191

Raven JA, Cockell CS, De La Rocha CL (2008) The evolution of inorganic carbon concentrating mechanisms in photosynthesis. Philos Trans R Soc Lond B 363:2641–2650

Reed RH, Stewart WDP (1983) Physiological responses of *Rivularia atra* to salinity: osmotic adjustment in hyposaline media. New Phytol 95:595–603

Reed RH, Stewart WDP (1988) The responses of cyanobacteria to salt stress. In: Rogers LJ, Gallon JR (eds) Biochemistry of algae and cyanobacteria. Clarendon, Oxford, pp 217–231, 374 pp

Reed RH, Borowitzka LJ, Mackay MA, Chudek JA, Foster R, Warr SCR, Moore DJ, Stewart WDP (1986a) Organic solute accumulation in osmotically stressed cyanobacteria. FEMS Microbiol Rev 39:51–56

Reed RH, Warr SRC, Kerby NW, Stewart WDP (1986b) Osmotic shock-induced release of low molecular weight metabolites from free-living and immobilized cyanobacteria. Enzyme Microbiol Technol 8:101–104

Rees DA (1969) Structure, conformation and mechanism in the formation of polysaccharide gels and network. Adv Carbohydr Chem Biochem 24:267–332

Reid RP, Browne KM (1991) Intertidal stromatolites in a fringing Holocene reef complex, Bahamas. Geology 19:15–18

Reid RP, Visscher PT, Decho AW, Stolz JF, Bebout BM, Dupraz C, Macintyre IG, Paerl HW, Pinckney JL, Prufert-Bebout L, Steppe TF, DesMarais DJ (2000) The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature 406:989–992

Reinhold L, Kosloff R, Kaplan A (1991) A model for inorganic carbon fluxes and photosynthesis in cyanobacterial carboxysomes. Can J Bot 69:984–988

Renstrom-Kellner E, Bergman B (1989) Glycolate metabolism in cyanobacteria. III. Nitrogen controls excretion and metabolism of glycolate in Anabaena cylindrica. Physiol Plant 77:46–51

Renstrom-Kellner E, Bergman B (1990) Glycolate metabolism in cyanobacteria. IV. Uptake, growth and metabolic pathways. Physiol Plant 78:285–292

Rentz JA, Kraiya C, Luther GW III, Emerson D (2007) Control of ferrous iron oxidation within circumneutral microbial iron mats by cellular activity and autocatalysis. Environ Sci Technol 41:6084–6089

Revsbech NP, Jørgensen BB, Blackburn TH, Cohen Y (1983) Microelectrode studies of the photosynthesis and O_2, H_2S and pH profiles of a microbial mat. Limnol Oceanogr 28:1062–1074

Richardson LL, Castenholz RW (1987a) Diel vertical movements of the cyanobacterium *Oscillatoria terebriformis* in a sulfide-rich hot spring microbial mat. Appl Environ Microbiol 53:2142–2150

Richardson LL, Castenholz RW (1987b) Enhanced survival of the cyanobacterium *Oscillatoria terebriformis* in darkness under anaerobic conditions. Appl Environ Microbiol 53:2151–2158

Richardson LL, Castenholz RW (1989) Chemokinetic motility responses of the cyanobacterium *Oscillatoria terebriformis*. Appl Environ Microbiol 55:261–263

Rippka R, Waterbury JB (1977) The synthesis of nitrogenase by non-heterocystous cyanobacteria. FEMS Microbiol Lett 2:83–86

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Risatti JB, Capman WC, Stahl DA (1994) Community structure of a microbial mat: the phylogenetic dimension. Proc Natl Acad Sci USA 91:10173–10177

Robbins LL, Blackwelder PL (1992) Biochemical and ultrastructural evidence for the origin of whitings: a biologically induced calcium carbonate precipitation mechanism. Geology 20:464–468

Robins RJ, Hall DO, Shi DJ, Turner RJ, Rhodes MJC (1986) Mucilage acts to adhere cyanobacteria and cultured plant cells to biological and inert surfaces. FEMS Microbiol Lett 34:155–160

Roeske CA, O'Leary M (1984) Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulose bisphosphate. Biochemistry 23:6275–6284

Romanowski G, Lorenz MG, Wackernagel W (1991) Adsorption of plasmid DNA to mineral surfaces and protection against DNAse-I. Appl Environ Microbiol 57:1057–1061

Rougerie F, Jehl C, Trichet J (1997) Phosphorus pathways in atolls: interstitial nutrient pool, cyanobacterial accumulation and Carbonate-Fluoro-Apatite (CFA) precipitation. Mar Geol 139:201–217

Ruff-Roberts AL, Kuenen JG, Ward DM (1994) Distribution of cultivated and uncultivated cyanobacteria and *Chloroflexus*-like bacteria in hot spring microbial mats. Appl Environ Microbiol 60:697–704

Schau L, Benson AA, Bassham JA, Calvin M (1950) The path of carbon in photosynthesis. XI. The role of glycolic acid. Physiol Plant 3:487–495

Schaub BEM, Van Gemerden H (1996) Sulfur bacteria in sediments of two coastal ecosystems: the Bassin d'Arcachon and the Étang du Prevost, France. Hydrobiologia 329:199–210

Schmetterer G (1994) Cyanobacterial respiration. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp 409–435, 881 pp

Schopf JW, Walter MR (1982) Origin and early evolution of cyanobacteria: the geological evidence. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 543–564, 688 pp

Serôdio J, Marques da Silva J, Catarino F (1997) Nondestructive tracing of migratory rhythms of intertidal benthic microalgae using *in vivo* chlorophyll *a* fluorescence. J Phycol 33:542–553

Severin I, Stal LJ (2008) Light dependency of nitrogen fixation in a coastal cyanobacterial mat. ISME J 2:1077–1088
Severin I, Acinas SG, Stal LJ (2010) Diversity of nitrogen-fixing bacteria in cyanobacterial mats. FEMS Microbiol Ecol 73:514–525
Siegesmund M, Johansen JR, Karsten U, Friedl T (2008) *Coleofasciculus* gen. nov. (Cyanobacteria): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. J Phycol 44:1572–1585
Smith AJ (1982) Modes of cyanobacterial carbon metabolism. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell, Oxford, pp 47–85, 688 pp
Smith RC, Prezelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, Macintyre S, Matlick HA, Menzies D, Ondrusek M, Wan Z, Waters KJ (1992) Ozone depletion – ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952–959
Srivastava P (2005) Vindhyan akinetes: an indicator of mesoproterozoic biosphere evolution. Orig Life Evol Biosph 35:175–185
Sroga GE (1997) Regulation of nitrogen fixation by different nitrogen sources in the filamentous non-heterocystous cyanobacterium *Microcoleus* sp. FEMS Microbiol Lett 153:11–15
Stal LJ (1993) Mikrobielle Matten. In: Meyer-Reil L-A, Köster M (eds) Mikrobiologie des Meeresbodens. Gustav Fischer, Jena, pp 196–220, 290 pp
Stal LJ (1994) Microbial mats in coastal environments. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 21–32
Stal LJ (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol 131:1–32
Stal LJ (2000) Cyanobacterial mats and stromatolites. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 61–120, 669 pp
Stal LJ (2001) Coastal microbial mats: the physiology of a small-scale ecosystem. S Afr J Bot 67:399–410
Stal LJ (2010) Microphytobenthos as a biogeo-morphological force in intertidal sediment stabilization. Ecol Eng 36:236–245
Stal LJ, Heyer H (1987) Dark anaerobic nitrogen fixation (acetylene reduction) in the cyanobacterium *Oscillatoria* sp. FEMS Microbiol Ecol 45:227–232
Stal LJ, Krumbein WE (1981) Aerobic nitrogen fixation in pure cultures of a benthic marine *Oscillatoria* (cyanobacteria). FEMS Microbiol Lett 11:295–298
Stal LJ, Krumbein WE (1985) Isolation and characterization of cyanobacteria from a marine microbial mat. Bot Mar 28:351–365
Stal LJ, Krumbein WE (1987) Temporal separation of nitrogen fixation and photosynthesis in the filamentous, non-heterocystous cyanobacterium *Oscillatoria* sp. Arch Microbiol 149:76–80
Stal LJ, Moezelaar R (1997) Fermentation in cyanobacteria. FEMS Microbiol Rev 21:179–211
Stal LJ, Reed RH (1987) Low-molecular mass carbohydrate accumulation in cyanobacteria from a marine microbial mat in response to salt. FEMS Microbiol Ecol 45:305–312
Stal LJ, Grossberger S, Krumbein WE (1984) Nitrogen fixation associated with the cyanobacterial mat of a marine laminated microbial ecosystem. Mar Biol 82:217–224
Stal LJ, Van Gemerden H, Krumbein WE (1985) Structure and development of a benthic marine microbial mat. FEMS Microbiol Ecol 31:111–125
Stal LJ, Paerl HW, Bebout B, Villbrandt M (1994) Heterocystous versus non-heterocystous cyanobacteria in microbial mats. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 403–414
Stal LJ, Behrens SB, Villbrandt M, Van Bergeijk S, Kruyning F (1996) The biogeochemistry of two eutrophic marine lagoons and its effect on microphytobenthic communities. Hydrobiologia 329:185–198
Steppe TF, Paerl HW (2002) Potential N_2 fixation by sulfate-reducing bacteria in a marine intertidal microbial mat. Aquat Microb Ecol 28:1–12
Steppe TF, Olson JB, Paerl HW, Litaker RW, Belnap J (1996) Consortial N_2 fixation: a strategy for meting nitrogen requirements of marine and terrestrial cyanobacterial mats. FEMS Microbiol Ecol 21:149–156
Steudel R, Holdt G, Visscher PT, Van Gemerden H (1990) Search for polythionates in cultures of *Chromatium vinosum* after sulfide incubation. Arch Microbiol 153:432–437
Steunou A-S, Bhaya D, Bateson M, Melendrez M, Ward D, Brecht E, Peters JW, Kühl M, Grossman A (2006) *In situ* analysis of nitrogen fixation and metabolic switching in unicellular thermophilic cyanobacteria inhabiting hot spring microbial mats. Proc Natl Acad Sci USA 103:2398–2403
Steunou A-S, Jensen SI, Brecht E, Becraft ED, Bateson MM, Kilian O, Bhaya D, Ward DM, Peters JW, Grossman AR, Kühl M (2008) Regulation of nif gene expression and the energetics of N_2 fixation over the diel cycle in a hot spring microbial mat. ISME J 2:364–378
Stolz JF (1993) Magnetosomes. J Gen Microbiol 139:1663–1670
Storch TA, Saunders GW, Ostrofsky ML (1990) Diel nitrogen fixation by cyanobacterial surface blooms in Sanctuary Lake, Pennsylvania. Appl Environ Microbiol 56:466–471
Straub KL, Benz M, Schink B, Widdel F (1996) Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. Appl Environ Microbiol 62:1458–1460
Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3–9
Tabita FR (1988) Molecular and cellular regulation of autotrophic carbon dioxide fixation in microorganisms. Microbiol Rev 52:155–189
Tago Y, Aida K (1977) Exocellular mucopolysaccharide closely related to bacterial floc formation. Appl Environ Microbiol 34:308–314
Taiz L (1984) Plant cell expansion: regulation of cell wall mechanical properties. Annu Rev Plant Physiol 35:585–657
Talbot MMB, Bate GC, Campbell EE (1990) A review of the ecology of surf-zone diatoms, with special reference to *Anulus australis*. Oceanogr Mar Biol Annu Rev 28:155–175
Tamagnini P, Axelsson R, Lindberg P, Oxelfelt F, Wünschiers R, Lindblad P (2002) Hydrogenases and hydrogen metabolism of cyanobacteria. Microbiol Mol Biol Rev 66:1–20
Tamagnini P, Leitão E, Oliveira P, Ferreira D, Pinto F, Harris DJ, Heidorn T, Lindblad P (2007) Cyanobacterial hydrogenases: diversity, regulation and applications. FEMS Microbiol Rev 31:692–720
Tease B, Jürgens UJ, Golecki JR, Heinrich UR, Rippka R, Weckesser J (1991) Fine-structural and chemical analyses on inner and outer sheath of the cyanobacterium *Gloeothece* sp. PCC-6909. Antonie Van Leeuwenhoek Int J Gen Mol Microbiol 59:27–34
Teske A, Ramsing NB, Habicht K, Fukui M, Küver J, Jørgensen BB, Cohen Y (1998) Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl Environ Microbiol 64:2943–2951
Trouwborst RE, Johnston A, Koch G, Luther GW III, Pierson BK (2007) Biogeochemistry of Fe(II) oxidation in a photosynthetic microbial mat: implications for Precambrian Fe(II) oxidation. Geochim Cosmochim Acta 71:4629–4643
Trüper HG, Galinski EA (1989) Compatible solutes in halophilic phototrophic procaryotes. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 342–348, 511 pp
Turner SM, Malin G, Liss PS, Harbour DS, Holligan PM (1988) The seasonal variation of dimethyl sulfide and dimethylsulfonio-propionate concentrations in nearshore waters. Limnol Oceanogr 33:364–375
Turpin DH, Miller AG, Canvin DT (1984) Carboxysome content of *Synechococcus leopoliensis* (Cyanophyta) in response to inorganic carbon. J Phycol 20:249–253
Utkilen HC (1976) Thiosulphate as electron donor in the blue-green alga *Anacystis nidulans*. J Gen Microbiol 95:177–180
Valladares A, Montesinos ML, Herrero A, Flores E (2002) An ABC-type, high-affinity urea permease identified in cyanobacteria. Mol Microbiol 43:703–715

Van Bergeijk SA, Stal LJ (1996) The role of oxygenic phototrophic microorganisms in production and conversion of dimethylsulfoniopropionate and dimethylsulfide in microbial mats. In: Kiene RP, Visscher PT, Keller MD, Kirst GO (eds) Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York, pp 369–379, 430 pp

Van Gemerden H (1987) Competition between purple sulfur bacteria and green sulfur bacteria: role of sulfide, sulfur and polysulfides. Acta Acad Abo 47:13–27

Van Gemerden H (1993) Microbial mats: a joint venture. Mar Geol 113:3–25

Van Gemerden H, Tughan CS, De Wit R, Herbert RA (1989) Laminated microbial ecosystems on sheltered beaches in Scapa Flow, Orkney Islands. FEMS Microbiol Ecol 62:87–102

Van Liere L, Mur LR (1979) Growth kinetics *of Oscillatoria agardhii* Gomont in continuous culture, limited in its growth by the light energy supply. J Gen Microbiol 115:153–160

Verrecchia EP, Dumant J-L, Collins KE (1990) Do fungi building limestone exist in semi-arid regions? Naturwissenschaften 77:584–586

Vila-Costa M, Simó R, Harada H, Gasol JM, Slezak D, Kiene RP (2006) Dimethylsulfoniopropionate uptake by marine phytoplankton. Science 314:652–654

Villbrandt M (1992) Interactions of nitrogen fixation and photosynthesis in marine cyanobacterial mats (Mellum, Southern North Sea). PhD thesis, University of Oldenburg, Oldenburg, Germany, 163 pp

Villbrandt M, Stal LJ (1996) The effect of sulfide on nitrogen fixation in heterocystous and non-heterocystous cyanobacterial mat communities. Algol Stud 83:549–563

Villbrandt M, Stal LJ, Krumbein WE (1990) Interactions between nitrogen fixation and oxygenic photosynthesis in a marine cyanobacterial mat. FEMS Microbiol Ecol 74:59–72

Vincent WF, Castenholz RW, Downes MT, Howard-Williams C (1993a) Antarctic cyanobacteria – light, nutrients, and photosynthesis in the microbial mat environment. J Phycol 29:745–755

Vincent WF, Downes MT, Castenholz RW, Howard-Williams C (1993b) Community structure and pigment organisation of cyanobacteria-dominated microbial mats in Antarctica. Eur J Phycol 28:213–221

Visscher PT (1992) Microbial sulfur cycling in laminated marine ecosystems. PhD thesis, University of Groningen, Groningen, 113 pp

Visscher PT, Van Gemerden H (1991) Production and consumption of dimethylsulfoniopropionate in marine microbial mats. Appl Environ Microbiol 57:3237–3242

Visscher PT, Van Gemerden H (1993) Sulfur cycling in laminated marine microbial ecosystems. In: Oremland RS (ed) Biogeochemistry of global change: radiatively active trace gases. Chapman and Hall, New York, pp 672–690, 879 pp

Visscher PT, Nijburg JW, Van Gemerden H (1990) Polysulfide utilization by *Thiocapsa roseopersicina*. Arch Microbiol 155:75–81

Visscher PT, Prins RA, Van Gemerden H (1992) Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat. FEMS Microbiol Ecol 86:283–293

Visscher PT, Reid RP, Bebout BM, Hoeft SE, Macintyre IG, Thompson JA (1998) Formation of lithified micritic lamninae in modern marine stromatolites (Bahamas): the role of sulfur cycling. Am Mineral 83:1482–1493

Visscher PT, Reid RP, Bebout BM (2000) Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. Geology 28:919–922

Vogt C, Rabenstein A, Rethmeier J, Fischer U (1998) Alkali-labile precursors of dimethyl sulfide in marine benthic cyanobacteria. Arch Microbiol 169:263–266

Walsby AE (1985) The permeability of heterocysts to the gases nitrogen and oxygen. Proc R Soc Lond B 226:345–366

Walter MR (ed) (1976) Stromatolites. Elsevier, Amsterdam, 790 pp

Walter MR, Heys GR (1985) Links between the rise of metazoa and the decline of stromatolites. Precambrian Res 29:149–174

Walter MR, Buick R, Dunlop JSR (1980) Stromatolites 3,400-3,500 Myr. old from the North Pole area, Western Australia. Nature 284:443–445

Walter MR, Grotzinger JP, Schopf JW (1992) Proterozoic stromatolites. In: Schopf JW, Klein C (eds) The proterozoic biosphere. A multidisciplinary study. Cambridge University Press, New York, pp 253–260, 1348 pp

Warburg O (1920) Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen.II. Biochem Z 103: 188–217

Warburg O, Krippahl G (1960) Glykolsäurebildung in *Chlorella*. Z Naturforsch B15:197–199

Ward DM, Weller R, Shiea J, Castenholz RW, Cohen Y (1989) Hot spring microbial mats: anoxygenic and oxygenic mats of possible evolutionary significance. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. ASM, Washington, DC, pp 3–15, 511 pp

Ward DM, Ferris MJ, Nold SC, Bateson MM, Kopcynski ED, Ruff-Roberts AL (1994) Species diversity in hot spring microbial mats as revealed by both molecular and enrichment culture approaches – relationship between biodiversity and community structure. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance. Springer, Heidelberg, pp 33–44, 463 pp

Warr SCR, Reed RH, Stewart WDP (1984) Osmotic adjustment of cyanobacteria: the effects of NaCl, KCl, Sucrose and glycine betaine on glutamine synthetase activity in a marine and a halotolerant strain. J Gen Microbiol 130:2169–2175

Warr SRC, Reed RH, Stewart WDP (1988) The compatibility of osmotica in cyanobacteria. Plant Cell Environ 11:137–142

Waterbury JB, Rippka R (1989) Subsection I. Order Chroococcales. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams and Wilkins, Baltimore, pp 1728–1746, 744 pp

Waterbury JB, Willey JM, Franks DG, Valois FW, Watson SW (1985) A cyanobacterium capable of swimming motility. Science 230:74–76

Westbroek P, Buddemeier B, Coleman M, Kok DJ, Fautin D, Stal LJ (1994) Strategies for the study of climate forcing by calcification. In: Doumenge F (ed) Past and presence biomineralization processes. Musée Oceanographique, Monaco, pp 37–60

Whale GF, Walsby AE (1984) Motility of the cyanobacterium *Microcoleus chthonoplastes* in mud. Br Phycol J 19:117–123

White AK, Metcalf WW (2007) Microbial metabolism of reduced phosphorus compounds. Annu Rev Microbiol 61:379–400

Whitton BA (1987) The biology of Rivulariaceae. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 513–534, 534 pp

Whitton BA, Potts M (1982) Marine littoral. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 515–542, 688 pp

Widdel F, Schnell S, Heising S, Ehrenreich A, Assmus B, Schink B (1993) Ferrous iron oxidation by anoxygenic phototrophic bacteria. Nature 362:834–836

Wieland A, Zopfi J, Benthien M, Kühl M (2005) Biogeochemistry of an iron-rich hypersaline microbial mat (Camargue, France). Microb Ecol 49:34–49

Wuichet K, Zhulin IB (2003) Molecular evolution of sensory domains in cyanobacterial chemoreceptors. Trends Microbiol 11:200–203

Yallop ML, De Winder B, Paterson DM, Stal LJ (1994) Comparative structure, primary production and biogenic stabilization of cohesive and non-cohesive marine sediments inhabited by microphytobenthos. Estuar Coast Shelf Sci 39:565–582Yechieli Y, Wood WW (2002)

Hydrogeologic processes in saline systems: playas, sabkhas, and saline lakes. Earth Sci Rev 58:343–365

Zavarzin GA, Gerasimenko LM, Zhilina TN (1993) Cyanobacterial communities in hypersaline lagoons of Lake Sivash. Microbiology 62:645–652

Zeebe RE, Wolf-Gladrow D (2001) CO_2 in seawater: equilibrium, kinetics and isotopes. Elsevier, New York, 346 pp

Zehr JP, Mellon M, Braun S, Litaker W, Steppe T, Paerl HW (1995) Diversity of heterotrophic nitrogen fixation genes in a marine cyanobacterial mat. Appl Environ Microbiol 61:2527–2532

Zehr JP, Bench SR, Carter BJ, Hewson I, Niazi F, Shi T, Tripp HJ, Affourtit JP (2008) Globally distributed uncultivated oceanic N_2-fixing cyanobacteria lack oxygenic photosystem II. Science 322:1110–1112

Marine Plankton

5

Hans W. Paerl

Contents

Summary

The marine environment, which includes estuarine, coastal and open ocean waters, is a phylogenetically rich repository of planktonic cyanobacteria. All major cyanobacterial groups are represented in the marine plankton, yet specific environmental constraints strongly select for certain groups to dominate in geographically and climatically distinct regions of the world's oceans. In this chapter, physical, chemical and biotic properties of estuarine, coastal and open ocean habitats are examined with respect to their controls on the diversity, abundance and distributions of marine planktonic cyanobacteria. The focus is on the filamentous and colonial cyanobacteria that periodically accumulate as dense "blooms" that may discolor oceanic and coastal waters. Blooms are of considerable biogeochemical and ecological significance, because they represent large concentrations of phytoplankton biomass that impact carbon, nutrient (N, P, Fe and micronutrients), and oxygen cycling. The smaller picoplanktonic forms are an additionally important biomass fraction addressed elsewhere (see Chap. 20 by Scanlan). Marine planktonic cyanobacteria employ a suite of morphological, physiological and ecological adaptations and strategies aimed at optimizing growth and reproduction in response to environmental constraints, including nutrient depletion (oligotrophy), variable degrees of turbulence, sub-optimal light and temperature conditions that characterize much of the world's oceans. These include N_2 fixation, nutrient sequestration and storage, buoyancy regulation, consortial and symbiotic associations, and coloniality. Specific planktonic taxa are able to exploit human and naturally-(climatic) induced environmental perturbations and changes, such as nutrient-enrichment, rising temperatures, increased tropical cyclone activity, altered rainfall patterns and droughts. Some cyanobacterial bloom taxa are considered harmful (CyanoHABs) because they can negatively affect water quality and habitat condition by producing toxins, exacerbating hypoxia, and altering food webs. Potential nutrient and other management strategies aimed at controlling CyanoHAB outbreaks and dominance are

H.W. Paerl (✉)
Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell Street, Morehead City, NC 28557, USA
e-mail: hpaerl@email.unc.edu

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addressed. The extent and limits of biotic evolution in this ancient group of metabolically-diverse phototrophs has strongly affected the geochemical and biotic changes characterizing the evolution of the Earth's oceans and biosphere.

5.1 Introduction

The marine environment, which includes waters ranging from brackish estuaries to the open ocean, spanning polar to tropical regions, is a rich repository of cyanobacterial diversity and biomass. To a large extent, this reflects the long evolutionary history of cyanobacteria, which were the first oxygenic phototrophs to inhabit the world's oceans, 3 + billion years ago (Schopf 1993; Knoll 2003). The advent and evolution of cyanobacteria in the marine environment ushered in some of its most profound and consequential biogeochemical and ecological changes; the most important of which was the transition from anoxic to oxic conditions. Ironically, while the monumental biotic "breakthrough" of oxygenic photosynthesis opened up vast segments of the world's oceans (and terrestrial environments) to the evolution of modern-day oxygen-requiring life forms, this major biospheric change also led to severe constraints on the process that brought it about; namely photosynthesis, as well as numerous other oxygen-sensitive metabolic and nutrient-transforming processes, including respiration and nitrogen fixation (Paerl 1990, 1988). This constitutes one of the great dilemmas in biotic evolution, and is of fundamental importance to understanding the current distribution, diversity and constraints on the proliferation and dominance of cyanobacteria in the world's oceans.

Much of the world's oceans can be characterized as a biological "desert", comprised of nutrient-deplete, ultraoligotrophic waters (Holland 1978; Capone et al. 2008; Paerl and Piehler 2008). As such, there is a premium on developing biotic strategies aimed at optimizing productivity and diversity for countering and circumventing nutrient-deprived conditions. Marine cyanobacteria evolved under these conditions, and it is argued that their morphological and ecophysiological diversification reflects these environmental limitations. Nutrient deficiency is only one of several environmental constraints that cyanobacteria face in the oceans. Their photosynthetic nature dictates that they reside in illuminated near-surface waters or the euphotic zone. These waters are also exposed to periodically-high levels of irradiance (including UV) and molecular oxygen, known to be a potential inhibitor of photosynthesis and key nutrient transformation processes, the most important of which is nitrogen fixation; the conversion of "inert" atmospheric N_2 to biologically-reactive ammonia (Paerl 1990, 1988). In addition, in order to sustain processes essential for biomass production (i.e. photosynthesis, nitrogen fixation), specific nutrients (e.g. P, Fe) are required. The availability of these nutrients may be severely restricted in the euphotic zone.

In this chapter, the linkage of these environmental constraints to the diversity, abundance and distributions of marine planktonic cyanobacteria will be explored. The focus will be on the filamentous and colonial cyanobacteria that periodically accumulate as dense "blooms" that can discolour oceanic and coastal waters. The smaller picoplanktonic forms, which are an important fraction of cyanobacterial and total phytoplankton biomass, are considered in Chap. 20. Morphological, physiological and ecological strategies employed to optimize growth and reproduction will be investigated. Additional focus will be on marine systems in which planktonic cyanobacteria are capable of exploiting human and naturally- (climatic) induced environmental perturbations and changes, such as nutrient-enrichment (eutrophication), rising temperatures, increased tropical cyclone activity, altered rainfall patterns and droughts. The overall objective is to examine and evaluate the advantages and limits of biotic evolution of this ancient group of phototrophs, which has "seen it all" with respect to geochemical and biotic evolutionary processes that have impacted and altered the marine environment.

5.2 Morphological and Ecophysiological Diversity of Marine Planktonic Cyanobacteria

Marine planktonic cyanobacteria are morphologically, physiologically and ecologically diverse. Cyanobacteria comprise a highly significant and at times dominant fraction of phytoplankton productivity and biomass (Fogg 1982; Paerl 2000; Capone et al. 1997). They exist as spheroid single or aggregated cells, aggregated or solitary non-heterocystous filamentous and heterocystous filamentous groups (Stanier and Cohen-Bazire 1977; Komarék and Anagnostidis 1986) (Fig. 5.1).

Morphologically, the simplest marine planktonic cyanobacterial group contains the single celled forms. They are also the most abundant, which is evident when examining a seawater sample microscopically or by flow cytometry with fluorescent excitation light. This group includes the non-N_2-fixing ubiquitous genera *Synechococcus*, *Chroococcus*, *Prochlorococcus* and *Synechocystis*, as well as a few recently-described N_2 fixing genera, including *Crocosphaera* (Rocap et al. 2002; Zehr et al. 2005; Moisander et al. 2010; Paerl et al. 2011; Bothe et al. 2011). Species in these genera consist of small, solitary oval to spherical cells, on the order of 1–3 μm in diameter for the non N_2 fixers, while *Crocosphaera* cells tend to be somewhat larger (up to 7 μm in diameter). The smaller (<3 μm) cyanobacterial group is also referred to as the "picoplankton" (see Chap. 20). The

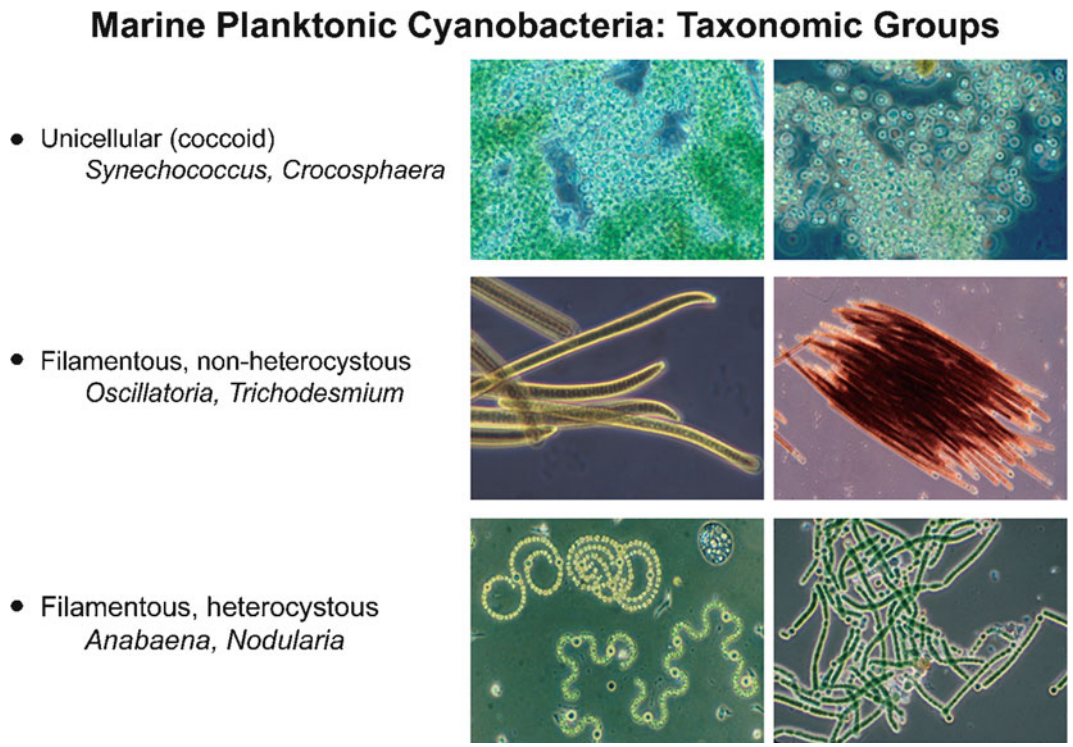

Fig. 5.1 Microphotographs of the major taxonomic groups and representative genera of marine planktonic cyanobacteria. Note that individual cells and filaments (trichomes) may be aggregated in colonies

picoplankters constitute an important fraction of the primary production and biomass (often in excess of 50%) of marine phytoplankton, especially in low nutrient, oligotrophic waters. However, they are not just confined to oligotrophic waters. Picoplanktonic cyanobacteria can also be a significant, and sometimes dominant, fraction of the phytoplankton in coastal and estuarine environments (Johnson and Sieburth 1979; Ray et al. 1989; Phlips et al. 1999; Marshall 2002; Murrell and Lores 2004). Lastly, unicellular cyanobacteria play a role as endosymbionts in diverse eukaryotic marine phytoplankton (Foster et al. 2006a, b; Escalera et al. 2010).

Because they are unicells, picoplanktonic cyanobacteria have morphological and physiological constraints on conducting oxygenic processes (e.g. O_2-evolving photosynthesis) and oxygen-sensitive processes (e.g. N_2 fixation) contemporaneously in the same cells. As a result, most oxygenic photosynthetic picoplanktonic genera are incapable of fixing N_2, although there are exceptions. A notable example is the genus *Gloeothece*, which is capable of N_2 fixation under dark conditions (at night), when photosynthesis is not active (Gallon 1992). Even at night, when oxygenic photosynthesis ceases, it is a challenge to fix N_2 in small single cells, because the ambient water column is usually fully oxygenated. Therefore, these diazotrophic genera must be able to offset inward diffusion of oxygen and maintain low intracellular oxygen concentrations through respiratory processes, and production of diffusive barriers (slimes and other polymeric substances). *Crocosphaera,* which is also capable of fixing N_2, (like *Gloethece* at night) was cultivated from Atlantic waters in the mid 1980s (Waterbury and Rippka 1989). Related strains have been cultivated from both the Atlantic and Pacific (Falcón et al. 2004; Zehr et al. 2001). This cyanobacterium is widely distributed and thus may be an important contributor to fixed C and N budgets of the world's oceans (Moisander et al. 2010).

The second group of marine planktonic cyanobacteria are the non-heterocystous, filamentous forms, which include the genera *Oscillatoria*, *Lyngbya*, *Leptolyngbya*, *Phormidium*, and *Spirulina*. Members of this group can be important contributors to estuarine and coastal planktonic and benthic primary production (Fogg 1982; Paerl 2000). Like the coccoid unicells, most of these undifferentiated filamentous cyanobacteria do not fix N_2. However, some species of *Lyngbya* and *Oscillatoria* are capable of diazotrophy, mostly during darkness and/or in oxygen-deplete microenvironments (aggregates, biofilms, mats, and as endosymbionts) (Zehr and Paerl 2008). This restricts their distributions (Paerl and Zehr 2000; Paerl and Kuparinen 2002). As such, they are generally not considered to be important contributors to large-scale estuarine, coastal or oceanic planktonic N_2 fixation budgets.

An important exception is the genus *Trichodesmium*, a widespread and ecologically important non-heterocystous N_2 fixer found throughout tropical and subtropical oceans (Capone et al. 1997; Karl et al. 2002). *Trichodesmium* is

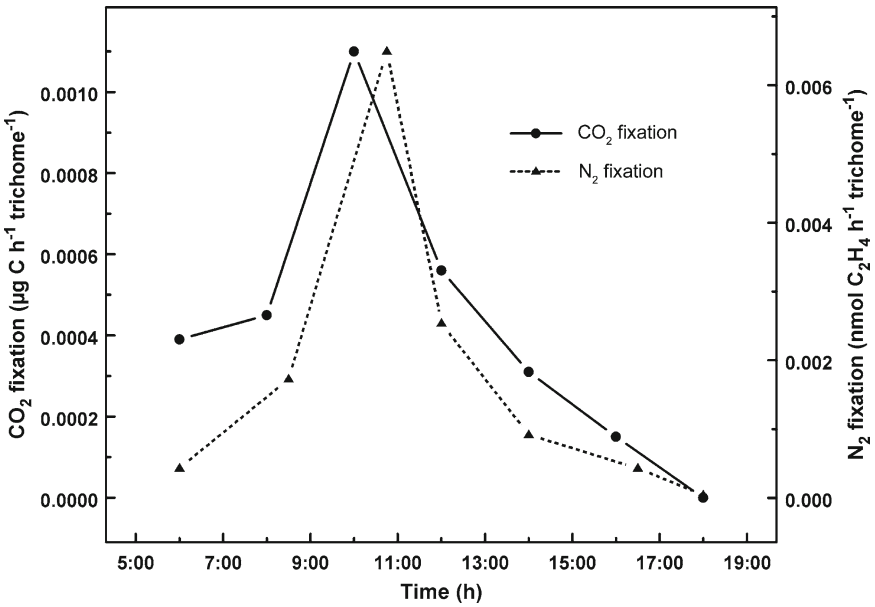

Fig. 5.2 Diel patterns of CO_2 fixation (as $^{14}CO_2$ incorporation) and N_2 fixation (nitrogenase activity, determined as rates of acetylene reduction) in natural populations of the ubiquitous sub-tropical/tropical planktonic *Trichodesmium* sp. Note that both activities co-occur and are confined to daytime, with CO_2 fixation tending to peak before N_2 fixation, a pattern also commonly observed in heterocystous cyanobacteria (Paerl 1990). Samples were freshly collected as part of a National Science Foundation supported Biocomplexity cruise in subtropical Pacific waters near Hawaii on 11 August 2003. They were incubated on shipboard (high intensity fluorescent lights) (Data provided courtesy of Dr Douglas Capone, University of Southern California, Los Angeles, CA)

capable of fixing N_2 during daytime; with patterns of N_2 fixation resembling those of oxygenic photosynthesis (light-mediated CO_2 fixation) (Fig. 5.2). This attribute not only distinguishes *Trichodesmium* from other non-heterocystous cyanobacterial diazotrophs; it is also a likely reason for its periodic dominance in N-deplete oligotrophic open ocean waters, where biologically-available N can be chronically deficient, but photosynthetically active radiation (PAR 400–700 nm) is plentiful.

Several hypotheses have been proposed to help explain the ability of *Trichodesmium* to fix N_2 in the light without heterocysts. One hypothesis is that there is combined temporal and spatial separation of activities (Berman-Frank et al. 2001), with reduced photosynthetic activities from the uncoupling of photosystem II (PS II) from photosystem I (PSI) occurring on short time scales that would allow N_2 fixation to occur (Küpper et al. 2004). However, evidence that cells conducting N_2 fixation do not photosynthesize has been elusive. It has also been proposed that the high respiration rates accompanying the Mehler reaction in *Trichodesmium* may provide respiratory protection of the O_2 sensitive nitrogenase complex (Berman-Frank et al. 2001; Kana 1993).

Spatial separation of these processes on either intra or intercellular levels has also been investigated (Paerl and Bebout 1988; Janson et al. 1993; Paerl 1994). Immunolocalization studies of the nitrogenase protein in *Trichodesmium* filaments and colonies have yielded contrasting results. Paerl et al. (1989) showed that most cells along filaments and throughout colonies were nitrogenase positive, indicating broad genetic potential for fixing N_2 throughout the colonies. On the other hand, El-Shehawy et al. (2003) and Fredriksson and Bergman (1995) indicated that there may be some differences in distribution of nitrogenase in cells along the filaments. They termed heavily-labeled (with the antibody to nitrogenase) cells "diazocytes". However, an inverse relationship between nitrogenase activity and PS II oxygen evolution in individual cells was not demonstrated. Paerl and Bebout (1988) and Paerl (1994) suggested that the partitioning (and hence coexistence) of these processes was more likely controlled by the development of oxygen gradients along the lengths and in aggregates of filaments.

The ability to form highly buoyant aggregates comprised of either bundled or radially-oriented filaments is a conspicuous trait of *Trichodesmium* (Fig. 5.3). These aggregates, also called "puffs" and "tufts", are the dominant forms in which *Trichodesmium* exists in surface blooms, originally called "sea sawdust" by Darwin (Seward 1909). Relatively high rates of photosynthesis and N_2 fixation have been associated these colonies (Carpenter and Capone 1992; Paerl and Bebout 1988; Paerl 1994, 1999). Hence, they are viable, active morphological forms of this diazotroph. Carpenter and Price (1976), and subsequently Paerl (1994) provided microautoradiographic evidence that *Trichodesmium* filaments arranged in aggregates showed spatial partitioning of the

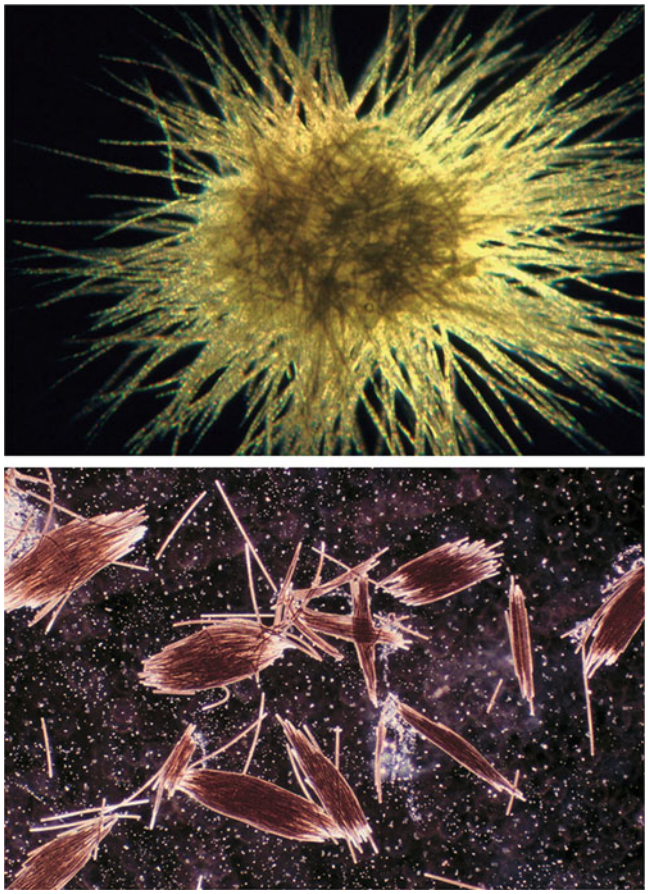

Fig. 5.3 Radially- ("puff") and fusiform- ("tuft") shaped aggregates of the non-heterocystous, filamentous N_2 fixing cyanobacterial genus *Trichodesmium*. Planktonic blooms of this organism can be comprised of just one form or a mixture of both

fixation of radiolabeled $^{14}CO_2$ along the lengths of filaments, with photosynthesis being most active in the outer distal regions. Paerl and Bebout (1988), using oxygen microelectrodes, demonstrated that in actively-photosynthesizing puff-shaped aggregates, the outer regions were highly oxygenated, while the inner, central regions were oxygen-deplete. This observation confirmed earlier findings that photosynthesis was most active in the outer regions of filaments and that the inner regions were relatively inactive (Carpenter and Price 1976). It also suggested that the tightly packed inner regions of aggregates may provide self shading, leading to relatively low rates of photosynthesis (and hence low rates of O_2 evolution). This would promote the formation of low-oxygen internal regions in which N_2 fixation could proceed in an uninhibited manner. Paerl (1994) also showed that in cultured *Trichodesmium* populations (strain IMS 101; Prufert-Bebout et al. 1993), individual filaments that were incubated with $^{14}CO_2$ revealed similar spatial partitioning of photosynthetic CO_2 fixation. However, unlike aggregates, which grew well over a range of light intensities up to 500 μmol photon m^2 s^{-1}, individual filaments failed to grow diazotrophically above light intensities greater than 100 μmol photon m^2 s^{-1}. This suggests that, when arranged in aggregates, *Trichodesmium* filaments laterally partition CO_2 and N_2 fixation and can co-optimize these potentially cross-inhibitory processes (Paerl 1994).

Models based on physical drivers (light, temperature, water circulation) of *Trichodesmium* blooms have been developed to help predict their distributions and magnitudes (Hood et al. 2001). *Trichodesmium* blooms are surface phenomena that lend themselves to being visualized from space; hence, remote sensing has proven to be a useful tool for spatially delineating and characterizing these blooms (Subramaniam et al. 2001). The model of Hood et al. (2001), which uses remote sensing imagery (Hood et al. 2002), is based on growth as a function of light, and predicts the distribution of *Trichodesmium* in the North Atlantic Ocean. This model predicts high abundances and N_2 fixation rates of *Trichodesmium* as part of successional events from upwelling regions (Hood et al. 2004). The contribution of *Trichodesmium* N_2 fixation to phytoplankton community N needs in the North Atlantic was calculated based on the relationships between remotely-sensed chlorophyll, sea surface height and temperature (SSH and SST). A model for the North Pacific incorporated light, temperature, phosphorus and wind stress, and the N_2 fixation rate was predicted from growth rate and elemental composition.

The heterocystous filamentous cyanobacteria are the third important functional group in the marine plankton. Heterocysts are differentiated vegetative cells that have "lost" the O_2 evolving component (photosystem II), but have retained photoreductive capabilities (photosystem I) of the photosynthetic apparatus (Gallon 1992; Zehr and Paerl 2008). These thick-walled cells, which are interspersed in filamentous taxa, are a highly-specific morphological and physiological adaptation to ambient oxic conditions, in that they harbour the oxygen-sensitive nitrogen (N_2) fixing apparatus in an O_2-free microenvironment (Wolk 1982). Fixed carbon and nitrogen compounds can be exchanged between heterocysts and neighboring vegetative cells (Wolk 1982). This enables heterocystous taxa to contemporaneously photosynthesize and fix atmospheric N_2, providing access to an unlimited supply of biologically-available N during the N-limited conditions that frequently characterize oxygen- and radiant-rich surface waters. On the face of it, heterocystous cyanobacteria appear to be the most highly evolved and well-adapted diazotrophs in aquatic environments; reflective of a modern-day oxygen-rich biosphere. Indeed, certain heterocystous genera (*Anabaena*, *Aphanizomenon*, *Nodularia*) can at times form dense surface blooms in some coastal sea environments, most notably the brackish Baltic Sea, and some coastal lagoonal ecosystems (Paerl and Fulton 2006). However, despite what appears to be a rather ingenious ecophysiological adaptation to oxic, N-deficient conditions,

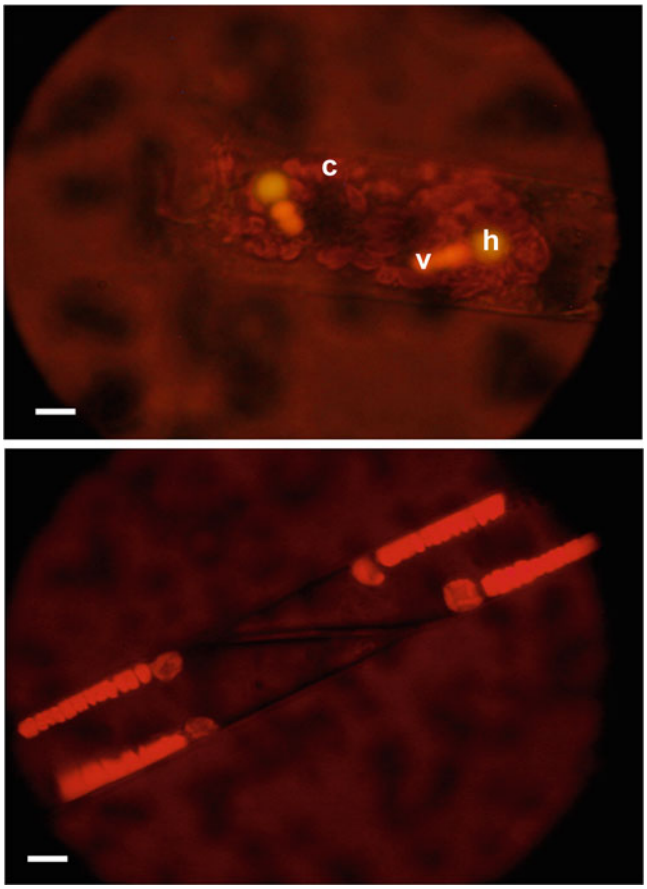

Fig. 5.4 Heterocystous cyanobacterial endosymbionts (cyanobionts) in marine planktonic diatoms. *Upper* Blue excitation and light epifluorescent micrograph of host diatom, *Hemiaulus membranaceus* associated with two *Richelia intracellularis* cyanobionts. The trichome of *Richelia* excites *yellow* (*h*, heterocyst) and *orange* (*v*, vegetative cells), while the chloroplast (*c*) of the *Hemiaulus* excites *red* under blue light (scale bar 5 μm). *Lower* Green excitation epifluorescent micrograph of the terminal end of two *Rhizosolenia* diatoms associated with two *Richelia intracellularis* cyanobionts. Phycobiliproteins excite *red* under green light as do the vegetative cells in *Richelia* (scale bar 5 μm). The images were taken during cruises to the subtropical N. Pacific (*upper*) and the Gulf of California (*lower*) (Images courtesy of Dr Rachel A Foster, Max Planck Institute for Marine Microbiology, Bremen, Germany)

it is puzzling why members of this group are not more common and dominant throughout the marine plankton, especially in chronically N deficient open ocean waters. Possible explanations for what appears to be an unfilled niche for these diazotrophs are discussed below.

Filamentous heterocyst-forming cyanobacteria can be found as endosymbionts in some open ocean diatom genera, including: *Rhizosolenia*, *Chaetoceros*, *Hemiaulus*, and *Bacteriastrom* (Fig. 5.4), but these species are exceedingly difficult to culture (Gómez et al. 2005; Janson et al. 1999; Villareal 1992, 1994) and hence our knowledge of their eco physiology is limited. Microscopic observations compared with molecular characterization studies revealed that the different symbiotic associations are comprised of phylogenetically distinct populations (Janson et al. 1999; Foster and Zehr 2006). Furthermore, quantitative PCR (QPCR) studies have shown that such associations are widely distributed, especially in tropical and subtropical regions (Church et al. 2005, 2006, 2008; Foster et al. 2008; Fong et al. 2008; Zehr et al. 2007). However, few direct measurements of CO_2 and N_2 fixation by these diatom-diazotroph associations have been made, mainly due to difficulty in their collection (c.f., Foster and O'Mullan 2008).

Taylor (1982) summarized anecdotal accounts of heterocystous *Anabaena* associated with *Coscinodiscus* diatoms, and Carpenter and Foster (2002) showed an epifluorescent micrograph of coccoid cyanobacteria within *Coscinodiscus* collected in the Indian Ocean near Zanzibar, Africa. Coccoid and small ovoid pico-cyanobacteria are most commonly associated with Dinophysoids (Dinoflagellates), Tintinnids and Radiolaria in the open sea (Foster et al. 2006a, b).

Additional heterocystous planktonic cyanobacterial genera/species have been reported in the + open ocean, including *Anabaena gerdii* (Carpenter and Janson 2001). Sightings of these free-living heterocystous cyanobacteria are rare. However, they are reported in geographically-diverse waters (albeit infrequently), including the Bermuda Atlantic Time-Series (BATS) station in the North Atlantic (Zehr et al. 2005) and the North Pacific gyre (Carpenter and Janson 2001). Reports of free-living *Richelia intracellularis*, the symbiont of the above mentioned diatoms, are infrequent with observations from the Pacific Ocean only (Gómez et al. 2005). Therefore, it is concluded that planktonic heterocystous cyanobacteria are widely distributed in the world's oceans, but they are most abundant as blooms in eutrophied seas and coastal systems and as endosymbionts in the open ocean, where they appear to live in protective diatom "glass houses".

5.3 Occurrence, Distribution and Environmental Controls on Marine Cyanobacteria

5.3.1 Occurrence and Distribution

Overall, cyanobacteria constitute an important component of marine phytoplankton communities (Fogg 1982; Paerl 2000). This is even more obvious when the picocyanobacteria are included (also see Chap. 18). Estimates of the fractions of oceanic phytoplankton attributable to cyanobacteria range from <10% in high latitude upwelling regions to >50% in tropical oligotrophic waters (Paerl 2000). Cyanobacteria also play important biogeochemical and trophic roles in coastal and estuarine waters. In the world's largest brackish water body, the Baltic Sea, extensive blooms of heterocystous filamentous genera (*Aphanizomenon*, *Anabaena*, *Nodularia*) occur during vertically-stratified summer months

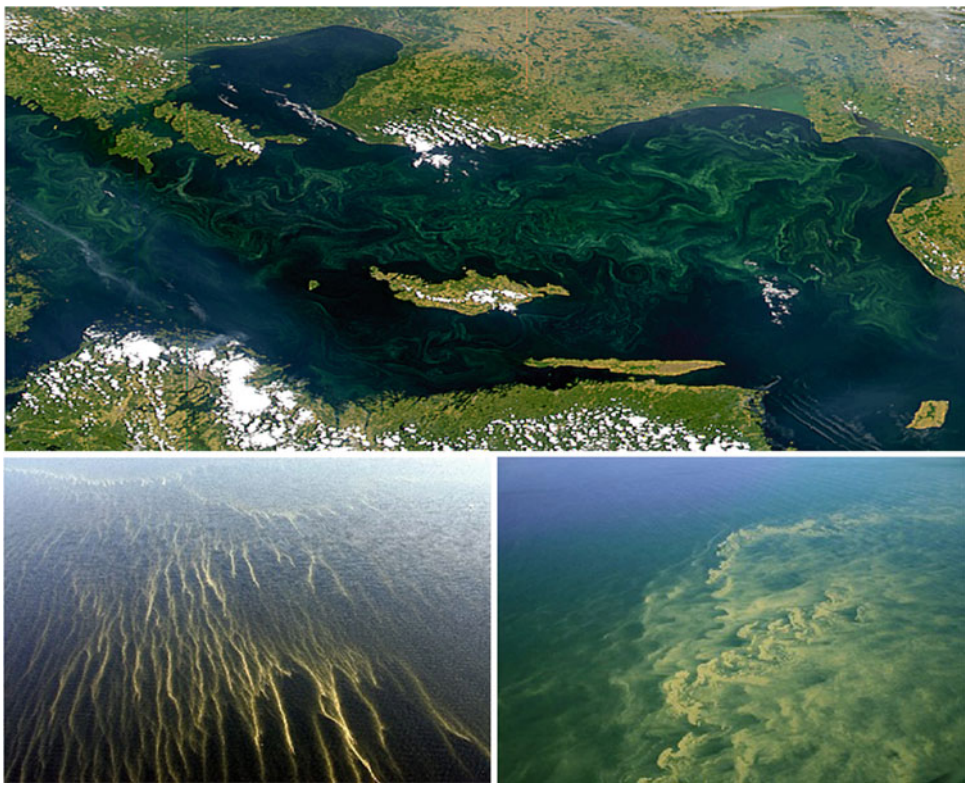

Fig. 5.5 Surface blooms of cyanobacteria in the Baltic Sea. *Upper frame*: A satellite (MODIS) image of blooms located between the Swedish and Finnish coasts near the entrance to the Gulf of Finland (Courtesy of NASA-MODIS Program). *Lower frames*: Aircraft imagery of the blooms in the Gulf of Finland (Courtesy of Finnish Border Guard). These blooms were comprised of the heterocystous N_2-fixing genera *Nodularia*, *Anabaena* and *Aphanizomenon*, as well as the coccoid non-N_2-fixing *Microcystis*

(Kononen et al. 1996) (Fig. 5.5). These blooms are significant sources of "new" N (~30% of "external" N inputs to the Baltic), and as such they play a central role in the N cycling and eutrophication dynamics of this system (Elmgren and Larsson 2001). Other examples include non- or micro-tidal lagoonal estuarine systems like the Peel-Harvey Estuary in Australia (*Nodularia*) (Huber 1986), Lake Ponchartrain, LA (*Anabaena*) (Dortch et al. 1999), sub-estuaries (Chowan, Neuse) of the lagoonal Pamlico Sound system, NC (Paerl 1983; Paerl et al. 2001), coastal lagoonal systems in Brazil and Colombia, South America, coastal western and southern Europe, and throughout Australasia (Paerl and Fulton 2006).

There appears to be a strong latitudinal gradient in cyanobacterial distribution and dominance, which can be attributed to several environmental factors, most notably ambient nutrient supplies and concentrations, and temperatures. In general, high nutrient, high latitude (both in the northern and southern hemispheres) ocean waters tend to be rich in large, fast growing eukaryotic phytoplankton taxa, including diatoms, and flagellates. In contrast, ultraoligotrophic tropical and subtropical waters, especially in semi-isolated gyre regions (e.g., N. Pacific Gyre, Sargasso Sea) tend to be dominated by smaller size phytoplankton, including picocyanobacteria, smaller filamentous cyanobacteria (e.g. *Phormidium*), and N_2 fixing cyanobacterial genera, most prominently *Trichodesmium*, as well as endosymbiotic heterocystous genera (e.g. *Richelia*) (Fogg 1982; Paerl 2000). The predominance of cyanobacteria in the plankton with decreasing latitudes closely follows increasing temperatures and decreasing nutrient availability (Paerl and Huisman 2009). In part, this reflects on the ability of diverse cyanobacterial genera to extract key growth-limiting nutrients (N, P, Fe) with high degrees of efficiency, using ecophysiological mechanisms not available to the eukaryotic phytoplankton with which they compete. These mechanisms include; (1) N_2 fixation, (2) highly efficient (low K_m) nutrient (N and P) uptake systems, (3) nutrient storage capabilities for P (polyphosphates) and N (phycobilins), (4) buoyancy regulation, which enables cells to periodically and rapidly "dip" into nutrient-rich deeper waters and then equally rapidly return to high-light near surface waters, and (5) symbiotic associations, which provide protection and enhance nutrient recycling within the "phycosphere" of the host eukaryotes (c.f. Paerl 1990).

Temperature also plays a major role in determining the geographic distributions of marine cyanobacteria. As a group, the cyanobacteria exhibit relatively slow growth rates and long doubling times when compared to eukaryotic taxa.

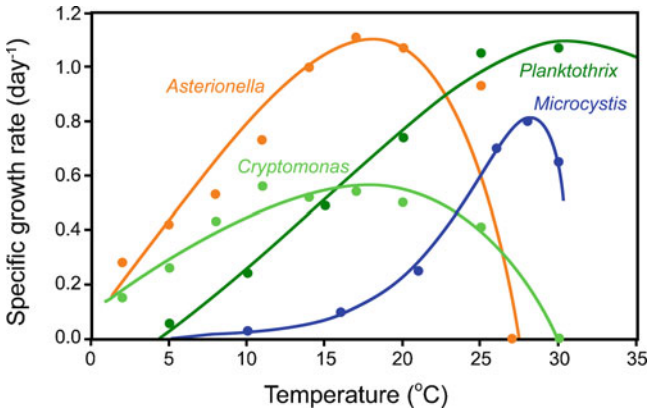

Fig. 5.6 Temperature dependence of the specific growth rates of *Microcystis aeruginosa* (Reynolds 2006) and *Planktothrix agardhii* (Foy et al. 1976), the diatom *Asterionella formosa* (Butterwick et al. 2005) and the cryptophyte *Cryptomonas marssonii* (Butterwick et al. 2005). The data are from controlled laboratory experiments using light-saturated and nutrient-saturated conditions. *Solid lines* are least-squares fits of the data to the temperature response *curve* of Chen and Millero (1986) (Figure adapted from Paerl and Huisman 2009)

This is most apparent at low temperatures (<10°C), where eukaryotic phytoplankton (i.e. diatoms, flagellates) growth rates are double to triple those of cyanobacteria, including picocyanobacteria. This temperature-growth differential becomes much smaller under warmer (>20°C) conditions (Peeters et al. 2007; Jöhnk et al. 2008; Paerl and Huisman 2009) (Fig. 5.6). In terms of distribution and dominance, cyanobacteria compete much better with eukaryotes, growth rate-wise, when warm water conditions prevail, as occur in the tropics. This begs the question as to whether cyanobacteria will compete more effectively in response to regional and global warming, a scenario known to benefit freshwater cyanobacterial bloom species (Paerl and Huisman 2008, 2009; Jöhnk et al. 2008). The influence of a warmer biosphere on cyanobacterial community composition, dominance and bloom potentials requires scrutiny, especially in estuarine and coastal waters influenced by anthropogenic nutrient enrichment and eutrophication (Paerl et al. 2011a, b). This human-climate interaction may be advantageous to the growth and proliferation of cyanobacteria in the marine environment.

The Baltic Sea offers a "looking glass" into this potential ecological scenario. This large brackish system exhibits a gradient in salinity ranging from near full seawater salinity at the entrance to the North Sea (the Kattegat and Skagerrak) to freshwater conditions in its northern lobe, the Bothnian Bay, and its easternmost region, the Gulf of Finland. Major segments of the Baltic Sea show strong vertical salinity and temperature stratification in summer months. This is accompanied by bottom water hypoxia and semi-permanent anoxic basins (Conley et al. 2009a). The Baltic Sea is surrounded by numerous Scandinavian, Western and Central European countries that have undergone expanding agriculture, urbanization and industrialization, accompanied by increases in nutrient loading over the past several centuries. Paleostratigraphic sediment evidence indicates that eutrophication has accompanied the period of enhanced nutrient loading (Elmgren 1989; Elmgren and Larsson 2001). In particular, the presence and proliferation of bloom-forming cyanobacteria has been a long-term phenomenon (since the 1800s) in the Baltic Sea (Bianchi et al. 2000). The combined effects of nutrient over-enrichment, periodic summer surface water heating and strong vertical stratification have provided ideal conditions for summer dominance by potentially toxic, bloom-forming heterocystous N_2 fixing (*Anabaena*, *Aphanizomenon*, *Nodularia*) and non-heterocystous non-N_2 fixing (*Microcystis*) cyanobacteria.

Nutrient (both N and P) input controls have been prescribed in efforts to reverse this unwanted situation (Elmgren 1989; Elmgren and Larsson 2001; Boesch et al. 2005), and some benefits of nutrient reductions have already been evident in parts of the system. For example, nutrient input reductions due to the collapse of agricultural production in the former Soviet Union eastern Baltic states in the late 1980s-early 1990s is thought to have been responsible for a reduction in noxious blooms of *Microcystis* and *Aphanizomenon* in the Estonian Bight and southern Baltic coastal waters bordering this region (Nausch et al. 1999), and N and P input reductions in the Gulf of Finland have likewise led to a reduction in the magnitude of cyanobacterial blooms there (Kirrikki et al. 2001). In Sweden, nutrient input reductions to fjords draining into the Baltic Sea have reduced algal bloom (including cyanobacterial) potentials (Elmgren and Larsson 2001).

It is possible that increased surface water temperatures, resulting from a recent period of warming in Northern Europe, may also be playing a role in the promotion of cyanobacterial blooms. For example, lakes in Central and Northern Europe that have experienced symptoms of warming (i.e. earlier periods of surface ice melting, higher surface water temperatures and stronger, more persistent vertical stratification) have shown a propensity for expansion of N_2 fixing (*Cylindrospermopsis*, *Anabaena*, *Aphanizomenon*) and non-N_2 fixing (*Microcystis*) cyanobacterial bloom species (Stüken et al. 2006; Wiedner et al. 2007). There is similar evidence that such a trend may be occurring in the Baltic Sea proper (Suikkanen et al. 2007). Therefore climate change, especially warming, may be playing an interactive (with nutrient over-enrichment) role in controlling cyanobacterial dominance in the plankton (Paerl and Huisman 2008, 2009; Paerl et al. 2011a, b).

Despite what appears to be man-made and climatic environmental changes that favor the expansion of cyanobacterial blooms, combined with the fact that N_2 fixing cyanobacteria enjoy an obvious advantage in N limited

marine ecosystems, only rarely do these diazotrophs dominate phytoplankton communities in N deplete estuarine, coastal and open ocean waters. Notable exceptions are extensive blooms of the non-heterocystous diazotroph *Trichodesmium* (Carpenter and Capone 1992; Capone et al. 1997; Karl et al. 2002) and associations between diatoms and diazotrophic cyanobacterial endosymbionts (e.g. *Rhozosolenia- Richelia*) in subtropical and tropical open ocean waters (Carpenter and Capone 1992; Carpenter and Foster 2002), and filamentous non-heterocystous (*Lyngbya*) and heterocystous (*Nodularia*) genera in nutrient-enriched and periodically-stratified brackish, lagoonal and periodically-stratified waters, where the combined effects of nutrient enrichment and water column stability appear to favor their growth (Paerl 1990; Paerl and Zehr 2000). To some extent, these taxa may be benefitting from man-made and climatic changes taking place on land masses bordering the subtropical oceans. One example is the desertification of Sub-Saharan Africa, a phenomenon that has been exacerbated by recent persistent droughts and deforestation (Bou Karam et al. 2009). These effects have increased the mobilization of iron and phosphorus as dust in the atmosphere during sandstorms. Dust clouds from these storms are advected over the Eastern Equatorial Atlantic, where they constitute important sources of "new" Fe and P in surface waters (Fig. 5.7). Buoyant *Trichodesmium* and other cyanobacterial populations that require these nutrients for diazotrophic growth benefit from these nutrient inputs (Walsh and Steidinger 2001; Capone et al. 2008). Therefore, there appears to be a mechanistic connection between increasing aeolian supplies of Fe and P, cyanobacterial growth and bloom potentials.

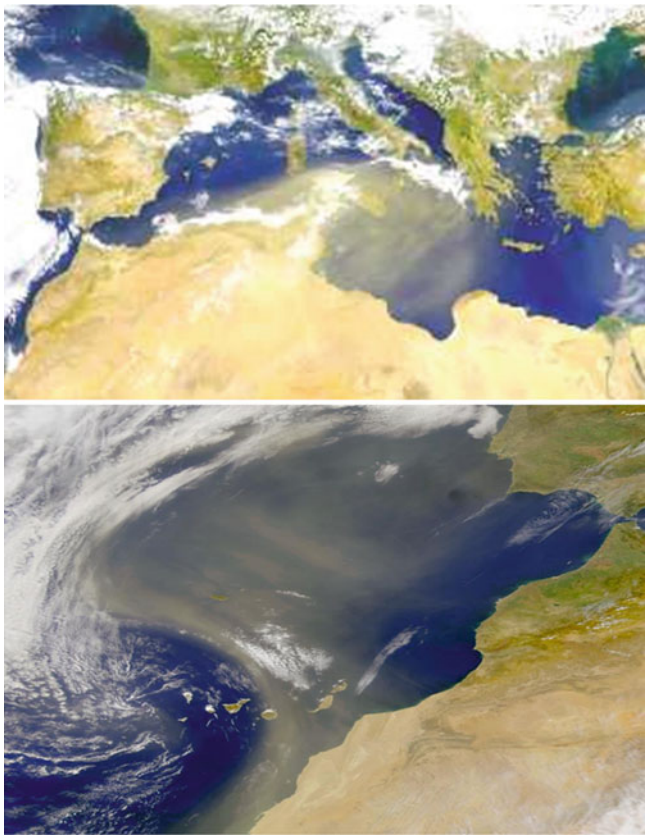

Fig. 5.7 Iron-rich dust clouds originating from the Sahara and sub-Sahara regions of Africa. These clouds, which can travel many hundreds of km and are advected over Fe-poor oligotrophic ocean waters, are considered important sources of "new" Fe supporting CO_2 and N_2 fixing requirements for surface dwelling cyanobacteria (e.g. *Trichodesmium*) populations. Shown here are dust emissions over the Mediterranean Sea and Eastern Atlantic Ocean (Photographs courtesy of NASA SeaWiFS Program)

5.3.2 Environmental Controls

From ecological and environmental perspectives, factors influencing cyanobacterial development, growth and expansion in marine planktonic habitats are complex and interactive. Furthermore, these factors are at the mercy of natural events, and changes in these events (such as droughts, floods, regional and global climate change), and human watershed-based activities (such as nutrient, other pollutant and sediment loadings). Potential regulatory factors include:

1. Nitrogen and phosphorus supply rates, ratios, chemical forms and sources.
2. Iron and trace metals.
3. Organic matter composition and concentrations.
4. Sedimentation and turbidity.
5. Salinity.
6. Light and temperature.
7. Water residence (flushing) times.
8. Water column vertical stratification and stability.
9. Biological interactions, such as microbial antagonism and synergism, and grazing.
10. Climate change (which is linked to many of the above factors).

A conceptual figure of physical, chemical and biotic factors influencing cyanobacterial growth and dominance is presented (Fig. 5.8). These factors interact in time and space. Identifying the mechanisms and effects of these interactions is critical to understanding controls of cyanobacterial growth and bloom formation.

5.3.2.1 Nitrogen and Phosphorus

Among the macronutrients required for aquatic plant growth and function in the marine environment, N and P are generally in shortest supply relative to need in that order (c.f. Redfield 1958, Dugdale 1967; Elmgren and Larsson 2001). Accordingly, enrichment with one or both of these nutrients usually stimulates growth (Vollenweider et al. 1992; Fisher et al. 1992; Paerl 2009). In marine ecosystems,

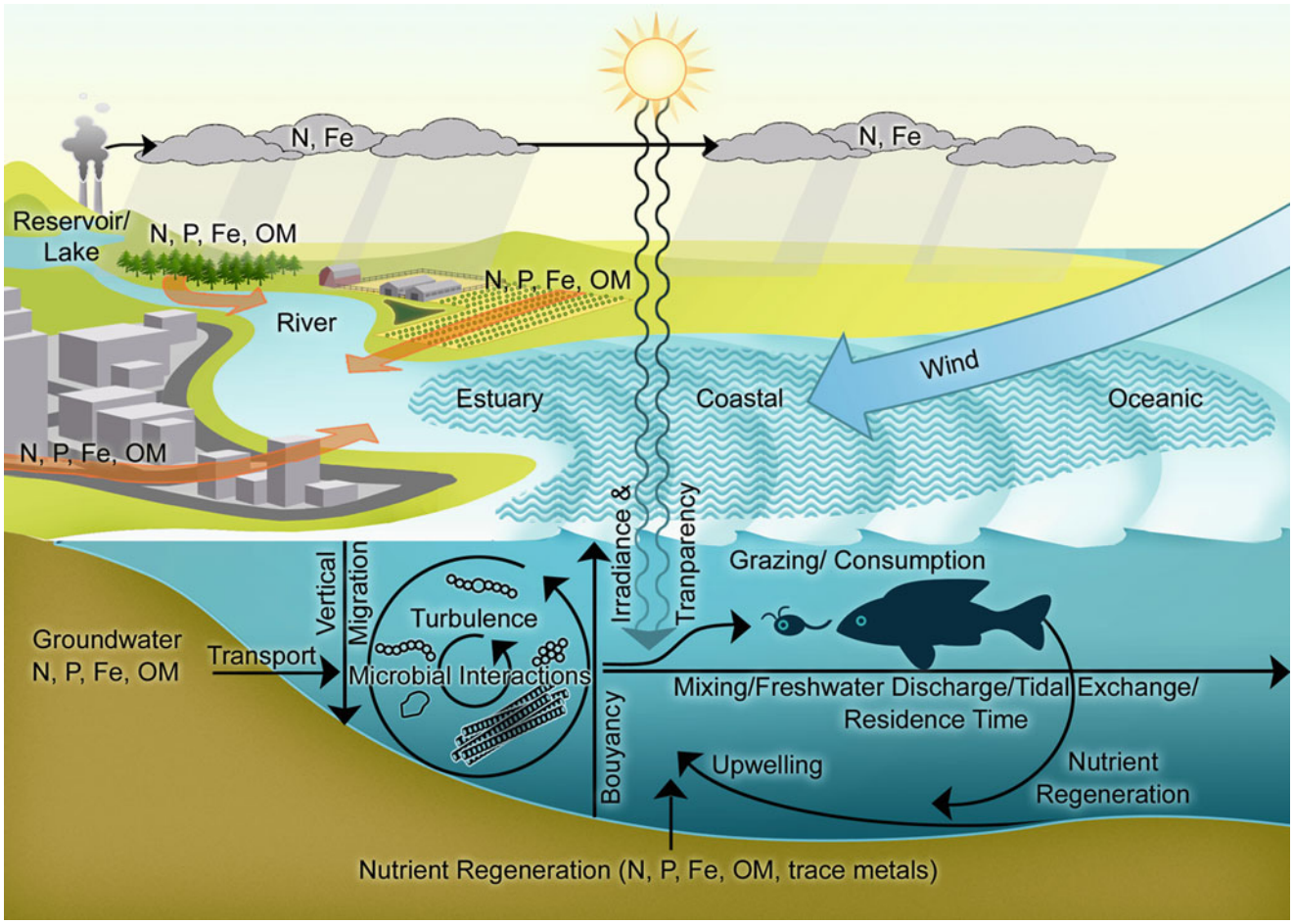

Fig. 5.8 Conceptual representation of the physical, chemical and biotic factors influencing marine planktonic cyanobacterial growth and dominance (Adapted from Paerl 2008)

eutrophication is most commonly referred to as nutrient-enhanced organic matter production (Nixon 1995). While adequate fertility is essential for ecosystem productivity and function, there are numerous negative impacts associated with nutrient over-enrichment, including harmful algal blooms, hypoxia, toxicity and food web alterations (Boesch et al. 2001; Rabalais and Turner 2001; Paerl and Piehler 2008). Cyanobacteria are among harmful algal bloom taxa able to exploit nutrient over-enrichment (Paerl 1988; Paerl and Millie 1996; Carmichael 1997).

While P has been identified as the most common limiting nutrient in freshwater ecosystems (Likens 1972; Schindler 1975), N inputs most often control "new" production and eutrophication in the marine environment (Nixon 1995; Boesch et al. 2001; Paerl and Piehler 2008). Estuarine systems tend to fall between these nutrient limitation "paradigms", with P limited conditions often characterizing the low salinity oligohaline (<5 psu) upstream segments, and N limitation typifying more saline (>5 psu) downstream waters (D'Elia et al. 1986; Fisher et al. 1992; Paerl and Piehler 2008). It has been pointed out that P enrichment, relative to N, may favour the development of N_2-fixing cyanobacterial taxa (Schindler 1975; Smith 1983, 1990), especially if the affected water body favours the development of cyanobacterial blooms for other reasons such as long residence time (low rates of flushing), surface water temperatures periodically exceeding 20°C, surface water stability and persistent vertical stratification (Reynolds and Walsby 1975; Paerl 1988; Shapiro 1990). While this scenario is particularly evident in freshwater systems (Smith 1983), some estuarine systems, like the brackish Baltic Sea and other oligohaline estuaries can also exhibit such a response, especially if they experience periods of physical stability (long residence times) and vertical stratification (Paerl 1988; Paerl and Fulton 2006). It should be pointed out, however, that exclusively high P (relative to N) loading is not always the only "trigger" for cyanobacterial growth and bloom formation. Estuarine and coastal systems that receive high N loads (especially increasing loads) can also show cyanobacterial dominance, in this case by non-N_2 fixing species (Paerl and Fulton 2006).

Whether or not N_2 fixers dominate depends on several co-occurring physical-chemical factors, including the availability of biologically utilizable N relative to P (Smith 1983; Paerl 1988; Downing et al. 2001). In some instances, however, the "N:P rule" is not applicable. These include; (1) highly eutrophic, or hypertrophic, systems in which *both* N and P loadings are very large (i.e., where N and P supplies may exceed the assimilative capacity of the phytoplankton), and (2) highly flushed (e.g. tidal) systems, in which the flushing rate exceeds the maximal growth or doubling rates of cyanobacteria, which typically are no faster than 1 day^{-1}. In highly enriched systems, N and P may be supplied in excess of algal growth requirements. Under these circumstances, factors other than nutrient limitation (e.g. light, vertical mixing, flushing rates/residence time, conductivity/salinity, trace element availability, grazing) may dictate algal community composition and activities. Here, N_2 fixation confers little if any advantage; hence non-N_2 fixing taxa (including eukaryotic taxa) will predominate.

N-enriched estuarine and coastal waters have experienced a recent upsurge in non-cyanobacterial harmful blooms (toxic dinoflagellates, chrysophytes and even diatoms) (Smetacek et al. 1991; Hallegraeff 1993; Richardson 1997; Paerl and Whitall 1999). Therefore, reducing N inputs merits considerable attention (Elmgren and Larsson 2001; Boesch et al. 2001; Conley et al. 2009b). However, care should be exercised in *only* reducing N without paying attention to P. Marine sediments are rich repositories of biogenically-deposited P, and in the shallow systems P is generally efficiently cycled between sediments and the water column. This ensures a readily available source of regenerated P, which could potentially support the growth and proliferation of N_2 fixing cyanobacteria.

In the deep open ocean, where P recycling from sediments can not readily replenish surface water P supplies, diazotrophic cyanobacterial bloom genera (*Trichodesmium*, *Richelia*) have been shown to be sensitive to P inputs (Sañudo-Wilhelmy et al. 2001; Karl et al. 2002). Therefore, there are regions in the world's oceans where P availability may control the ability of these diazotrophic genera to meet ecosystem N requirements,

While diazotrophic cyanobacteria enjoy the advantage of subsistence on atmospheric N_2, they also thrive on various forms of combined N (including *both* inorganic and organic forms) (Paerl 1988). This nutritional flexibility may provide a key competitive advantage in response to anthropogenic N loading. Large pulses of non-point source N loading from atmospheric deposition and runoff have increased markedly and may be key drivers of freshwater and marine eutrophication (Nixon 1995; Vitousek et al. 1997; Paerl 1997, 1988). Cyanobacterial growth and bloom responses in N-limited North Carolina estuaries closely track (in time and space) such events (Pinckney et al. 1997). In particular, organic N and ammonium-enriched conditions may favour cyanobacteria (Pinckney et al. 1997) and toxicity of bloom genera (Paerl and Millie 1996). Previous observations of such correlations in nature (Pearsall 1932; Fogg 1969) have been largely overlooked.

In low salinity waters (<10 psu) non-N_2-fixing cyanoHAB genera, including *Microcystis*, *Lyngbya*, and *Oscillatoria*, can also exploit these N loading scenarios. These genera thrive under relatively low N:P ratios, provided adequate P supplies exist (Smith 1990, Paerl 1990). Estuaries with relatively abundant P supplies (either naturally or anthropogenically) and growing (non-point) N inputs are potential targets for these genera. In addition, systems susceptible to bottom water anoxia accompanied by sediment N and P release events may be vulnerable.

Organic forms of N and P may play an additional role in supporting and enhancing marine cyanobacterial growth. As a group, cyanobacteria are capable of utilizing dissolved organic N (DON) and P (DOP) (Paerl 1988; Antia et al. 1991). Therefore, selective enrichment with these compounds, especially in the absence of inorganic N and P availability, may select for cyanobacteria. This scenario seems most likely in response to DON enrichment, since receiving waters tend to be N limited. DOP may play a more important role as a nutrient source in open ocean environments, where inorganic P availability is most constrained. Overall, the roles of organic N and P sources in supporting marine phytoplankton growth and in potentially selecting for cyanobacteria capable of DON and DOP utilization need further investigation.

5.3.2.2 Iron

Iron (Fe) and a variety of trace metal micronutrients are essential for cyanobacterial growth. Fe plays a central role in mediating growth because it is necessary for the synthesis and activities of key enzymes involved in photosynthesis, electron transport, energy transfer, and N transformations (i.e. nitrate and nitrite assimilation, N_2 fixation). Iron limitation of phytoplankton growth has been demonstrated in open-ocean and coastal waters capable of supporting cyanobacterial blooms (e.g. *Trichodesmium*, *Rhizosolenia-Richelia* symbiosis) (Martin et al. 1994; Paerl et al. 1994, 1999). Iron limitation does not appear to be common in estuarine ecosystems, possibly because of Fe-enriched runoff, periodic hypoxia and anoxia (which would enhance sediment Fe release) and close sediment-water column biogeochemical coupling in these shallow systems. However, vast portions of the oligotrophic open ocean removed from land masses and hence sources of "new" Fe show "excess" N and P concentrations (relative to phytoplankton production rates) and Fe limited phytoplankton growth (Martin et al. 1994). In these waters, N_2 fixation and diazotrophic growth may also be mediated by Fe availability. Both near-shore (Gulf Stream) and offshore (Sargasso Sea) populations of *Trichodesmium* spp. exhibited Fe-limited rates of primary production and N_2

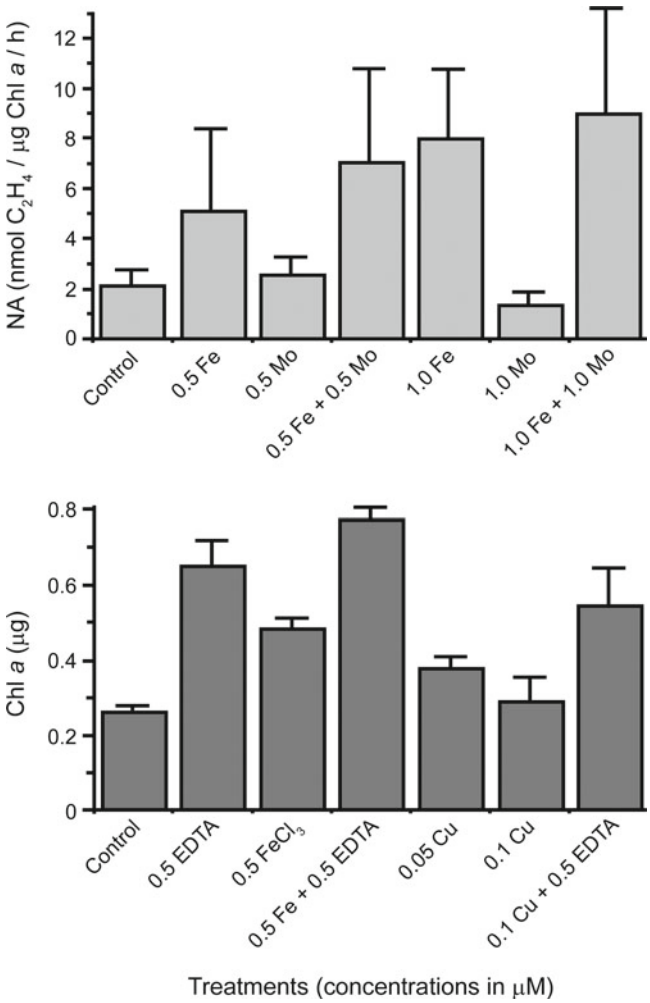

Fig. 5.9 Nutrient enrichment effects on the subtropical/tropical marine pelagic bloom-forming cyanobacterial genus *Trichodesmium*. A *Trichodesmium* bloom was detected in Western Atlantic Gulf Stream waters approximately 30 km off the coast of North Carolina, USA (1997). This bloom was collected and examined for nutrient limitation, on shipdeck under natural irradiance and temperature conditions, using bioassays as described in Paerl et al. (1994). N_2 fixation (nitrogenase activity *NA*) and growth (as chlorophyll *a*) were examined, relative to untreated controls, 48 h after nutrient additions. Responses to iron (Fe, as either EDTA-chelated or unchelated $FeCl_3$), molybdenum (Mo, as Na_2Mo_3), and copper (as $CuCl_2$) were examined in this bioassay

fixation in the W. Atlantic Ocean off the North Carolina coast (Paerl et al. 1994) (Fig. 5.9). Under defined culture conditions, *Trichodesmium* showed a relatively high requirement for this metal, which was not being met in open-ocean environments (Rueter et al. 1992). Growth and proliferation of non-diazotrophic marine phytoplankton, including bloom-forming species, may also be modulated by Fe availability. Modeling studies off Barbados (W. Atlantic, Caribbean Sea) suggested a link between Fe input and *Trichodesmium* blooms in that region (Lenes et al. 2005). In addition, the Florida red tide dinoflagellate *Gymnodinium breve*, which is often accompanied by *Trichodesmium* (as an N source), appeared controlled by Fe inputs from atmospheric (Saharan dust) and riverine discharge sources (Walsh and Steidinger 2001) (Fig. 5.7).

A morphological feature of buoyant *Trichodesmium* puff and tuft aggregates is their tendency to form peripheral regions of loosely packed filaments, in stark contrast to the tightly-packed, dense central regions (Fig. 5.3). It is hypothesized that the feathery external regions may serve as a "web" or "net" to trap iron-rich dust particles (Rueter et al. 1992) generated from upwind from desert storms (Sahara region in Africa) (Fig. 5.7). In this manner, N_2-fixing *Trichodesmium* aggregates are ensured ready access to atmospheric iron supplies in an environment normally considered depauperate of this nutrient.

Some bloom-forming cyanobacteria produce potent siderophore (hydroxamate) chelators, capable of sequestering iron at low ambient concentrations (Neilands 1967). This characteristic may provide a competitive advantage over eukaryotic phytoplankton when Fe availability is restricted (Murphy et al. 1976). Such a strategy would enable cyanobacteria to exploit Fe-limited waters. Bloom species may themselves affect Fe availability by mediating ecosystem productivity (i.e. organic matter production), bottom water hypoxia and anoxia, which can lead to the liberation of significant amounts of Fe, as the soluble reduced form Fe^{2+}. Lastly, by altering their bouyancy (Konopka et al. 1978; Reynolds 1987), bloom species are able to periodically "dip" into oxygen-deplete, Fe-rich bottom waters, thereby replenishing their Fe requirements. This strategy would be most relevant in eutrophic estuarine and coastal waters exhibiting bottom water hypoxia or anoxia. Most cyanobacterial bloom species are able to tolerate hypoxic and anoxic conditions by being insensitive to potentially-toxic hydrogen sulfide that builds up under these conditions.

Given possible ecophysiological strategies that enable cyanobacteria access to Fe under potentially limiting conditions, it is curious, if not puzzling, why cyanobacteria have not exerted greater dominance in Fe-limited waters. It is likely that relative to the impacts of excessive P loading (and low N:P ratios) and physical constraints discussed below, Fe limitation likely plays a secondary role in determining the distributions and magnitudes of cyanobacterial blooms. In this regard, it is interesting to note that recent schemes to increase primary production and thereby sequester atmospheric CO_2 by adding Fe to Fe-deplete but N and P replete oceanic waters (Boyd et al. 2007) may affect the relative dominance and function of cyanobacteria in oceanic phytoplankton communities.

5.3.2.3 Trace Metals

Cyanobacteria require a suite of trace metals for various metabolic, growth and reproductive processes (Allen and Stanier 1968). Photosynthesis and N_2 fixation require manganese,

zinc, cobalt, copper and molybdenum for synthesis and function (Holm-Hansen 1964). Copper (Cu), while toxic at >1 μM concentrations, is required for photosynthesis, energy transferring processes and cell growth. While this metal is present in most waters, its biologically available form, the Cu^{2+} ion, may be strongly bound by organic ligands, including humic and fulvic substances. Bioassays conducted on the cyanobacteria-dominated Chowan River, North Carolina, a brackish coastal river receiving heavy humic and fulvic acid loads from surrounding swamps and wetlands and from pulp mill effluent, showed that submicromolar additions of Cu (as $CuSO_4$) were capable of stimulating photosynthetic CO_2 fixation relative to untreated controls during periods of high humic discharge (Paerl 1983). During low humic discharge period, Cu stimulation of productivity was not observed. These results indicate that in the presence of high organic matter chelation capacity, Cu and possibly other chelatable metal (Fe?) limitations may exist in nature.

Molybdenum is a key cofactor in nitrogenase, the enzyme mediating N_2 fixation. Its availability could therefore play a key role in controlling the activity of diazotrophic cyanobacteria. Howarth and Cole (1985) proposed that the relatively high (>20 mM) concentrations of sulfate (SO_4^{2-}), a structural analogue of the most common form of molybdenum found in seawater, molybdate (MoO_4^{2-}), could competitively (via the uptake process) inhibit N_2 fixation, thereby controlling this process. Competitive inhibition of MoO_4^{2-} uptake by high SO_4^{2-} concentrations was shown experimentally by Cole et al. (1993). However, MoO_4^{2-} is highly soluble in seawater with concentrations on the order of ≈100 μM. Ter Steeg et al. (1986) and Paulsen et al. (1991) showed that, despite the potential for SO_4^{2-} competition, Mo was available at concentrations much lower than 100 μM. In both coastal and pelagic ocean (W. Atlantic) waters, N_2 fixing potentials of marine diazotrophs appear unaffected by this competition (Paulsen et al. 1991). Most likely, the small cellular Mo requirements for N_2 fixation are met though reduced but sufficient uptake and storage. In addition, "alternative" non Mo-requiring nitrogenases exist in bacterial and cyanobacterial diazotrophs (Bishop and Premakumar 1992). If such microbes are broadly distributed in nature, it would represent a mechanism by which Mo limitation could be circumvented regardless of ambient Mo concentrations.

Trace metal addition experiments with either natural or cultured populations have indicated that, in general, naturally-occurring concentrations of trace metals satisfy growth demands. It is therefore unlikely that trace metal limitation is a widespread phenomenon or modulator of cyanobacterial growth and bloom potentials in the marine planktonic environment. However, trace metals could play important synergistic roles with major nutrients (N, P) in determining phytoplankton species competitive interactions, and ultimately community composition.

5.3.2.4 Dissolved Organic Matter

Dissolved organic matter (DOM) has been mentioned as a possible modulator of cyanobacterial growth and bloom potential. Early studies (c.f. Fogg 1969) cite DOM as a factor potentially controlling cyanobacterial blooms. The hypothesized mechanism for DOM-stimulated cyanobacterial growth is that DOM "conditions" the water for cyanobacteria, possibly by inducing nutrient assimilatory enzymes and heterotrophy (Antia et al. 1991) or acting as nutrient (Fe and other trace metal) chelators, and providing a source of energy and nutrition for closely-associated heterotrophic bacteria, which may form synergistic interactions with cyanobacteria (Paerl and Pinckney 1996). Furthermore, as pointed out by Fogg (1969) and others (c.f. Lange 1967, Walsby 1974, Paerl 1990), elevated DOM may be a result (due to DOM excretion, bacteria and viral lysis, and "sloppy feeding" on cyanobacteria by grazing zooplankton), rather than a cause of cyanobacterial blooms. In addition, terrigenous organic (humic) substances discharged to coastal waters can strongly regulate phytoplankton activity, growth and composition. For example, these substances have been shown to interact with N, P and Fe availability to determine growth potential and potential dominance of the N_2 fixing toxin-producer *Nodularia spumigena* common to the Baltic Sea (Panosso and Granéli 2000).

5.3.2.5 Salinity

A highly distinctive chemical characteristic of seawater is elevated (relative to freshwater) salinity, i.e. ionic composition and strength. A noticeable biotic difference between freshwater and marine ecosystems is the much more widespread presence of cyanobacterial bloom species in freshwater systems. Furthermore, in systems that transition from freshwater to estuarine and coastal conditions (e.g. Scandinavian Fjords such as Himmerfjärden, Sweden, North American river-estuaries, including California's Klamath River, Florida's St Johns River, North Carolina's Neuse River and Maryland's Potomac River systems, Brazilian, Colombian, and Australian riverine-lagoonal estuarine systems), the upstream freshwater components can support large cyanobacterial blooms, while more saline downstream waters are often bloom-free. This suggests that increasing salinity may be a potential barrier to the growth and proliferation of cyanobacteria. Certain freshwater cyanobacterial bloom taxa, including the cosmopolitan non-N_2 fixer *Microcystis* and members of the diazotrophic genera *Anabaena*, *Aphanizomenon* and *Cylindrospermopsis* appear sensitive to elevated levels of salt (>2 psu) (Moisander and Paerl 2000; Moisander et al. 2002a), although some salt-tolerant species of *Microcystis* and *Anabaenopsis* have been isolated (Moisander et al. 2002a, b; Tonk et al. 2007). Experimental work has shown that when freshwater or soil-derived cyanobacterial populations are introduced into estuarine or coastal waters, they cannot compensate for increasing

salinities and osmotic stress (Paerl et al. 1983; Moisander et al. 2002a). Most of these taxa appear unable to adjust their osmotic potential (Du Bois and Kapusta 1981; Leredulier et al. 1984). Indigenous populations, however, are often able to adjust to varying salinities by the production of compatible osmolytes (Reed et al. 1986), so salinity sensitivity or adaptability to elevated salinity (including hypersalinity) appears to be taxa-specific among cyanobacteria.

Given the ability of some planktonic cyanobacterial bloom genera (*Anabaenopsis*, *Nodularia*, *Trichodesmium*) to thrive in full-salinity estuarine, coastal and oceanic waters, it is evident that salinity *per se* is not a barrier to the establishment and proliferation of cyanobacteria. Furthermore, estuarine and coastal environments worldwide support a diverse suite of benthic coccoid, non-heterocystous and heterocystous filamentous cyanobacteria, and in the case of diazotrophs, relatively high rates of N_2 fixation have been documented (Capone 1983; Paerl 1990). Oceanic reef, shelf, coastal mangrove, mudflat and hypersaline lagoonal habitats are particularly good habitats for diverse non-heterocystous (e.g. *Oscillatoria, Lyngbya, Microcoleus*) and heterocystous (e.g. *Scytonema, Nostoc, Anabaena, Cylindrospermum*, *Calothrix*) N_2-fixing and non-fixing genera (Potts 1980, 1994). A wide variety of diazotrophic and non-diazotrophic strains and species have been isolated from marine sediments, suspended aggregates, suspended plants and animals, pointing to the presence of a diverse cyanobacterial community in the marine environment (Paerl 1986, 1990, 1999). It is striking, however, that virtually all examples cited above are benthic or otherwise attached. Therefore, while it can be demonstrated that factors other than salinity control the diversity, distribution, and abundance of cyanobacteria in the marine environment, there appear to be more constraints on planktonic than benthic taxa. Grazing pressure does not explain the general lack of planktonic cyanobacterial dominance in nutrient-enriched estuarine and coastal waters (Paerl et al. 2001). However, as discussed below, physical constraints, including excessive turbulence, persistent vertical mixing and high rates of flushing (i.e. short residence time) that characterize many of the world's estuarine and coastal ecosystems, may exert strong and at times persistent controls on these otherwise opportunistic genera.

5.3.2.6 Temperature

Marine planktonic cyanobacteria are present over a wide spectrum of temperatures, ranging from Arctic and Antarctic (<4°C) to tropical ocean waters (>20°C). However, in terms of activity and growth, they generally exhibit optimal growth rates at relatively high temperatures, usually in excess of 25°C (Fogg 1956; Robarts and Zohary, 1987; Reynolds 1987). At these elevated temperatures, cyanobacteria compete most effectively with eukaryotic phytoplankton (diatoms, dinoflagellates, cryptophytes) (Butterwick et al. 2005; Elliott et al. 2006; De Senerpont Domis et al. 2007; Jöhnk et al. 2008). At such temperatures, growth rates of these eukaryotic taxa tend to level off or decline, while cyanobacterial growth rates reach their optima (Fig. 5.6). Furthermore, warming of surface waters intensifies vertical stratification.

Cyanobacterial bloom genera are able to uniquely exploit strongly-stratified surface waters. In contrast to eukaryotic phytoplankton, many of the bloom-forming genera, including heterocystous *Nodularia*, *Aphanizomenon*, *Anabaenopsis*, and *Anabaena* and the non-heterocystous genus *Trichodesmium* can regulate their vertical position in strongly stratified water (Villareal and Carpenter 1990), by counterbalancing the buoyancy of cellular gas vesicles by the accumulation of ballast in the form of carbohydrates (Walsby 1974). This enables bloom-forming taxa to migrate vertically, periodically obtaining nutrients from deeper waters, while subsequently returning to the surface as blooms (Huisman et al. 2005). These surface blooms take advantage of high levels of irradiance at the water surface to support their photosynthetic needs (Paerl 1988, 1990). Cyanobacterial bloom taxa contain photoprotective accessory pigments (e.g., carotenoids, scytonemins) that ensure long-term survival under extremely high irradiance conditions (Paerl et al. 1983). Dense surface blooms cast shade upon non-buoyant eukaryotic phytoplankton deeper down in the water column, thereby suppressing their competitors (Paerl 1988) (Fig. 5.10).

Evidence is mounting that the effects of regional and global warming, including stronger, more intensive and longer lasting vertical stratification, may be promoting the development, duration and geographic expansion (distributions) of buoyant, dwelling cyanobacterial blooms (Suikkanen et al. 2007; Wiedner et al. 2007; Paerl and Huisman 2008, 2009); in large part because these bloom species are able to take advantage of enhanced stratification and higher temperatures to support optimal growth (Peeters et al. 2007; Paerl and Huisman 2009; Paerl et al. 2011a, b) (Fig. 5.10).

Dense surface blooms of cyanobacteria may themselves locally increase water temperature, through the intense absorption of light by their photosynthetic and photoprotective pigments. In remote sensing studies, Kahru et al. (1993) found temperatures of surface blooms in the Baltic Sea to be at least 1.5°C above ambient waters. This represents a positive feedback mechanism, because by increasing their ambient temperatures, cyanobacteria gain a competitive advantage over eukaryotic phytoplankton.

One rather perplexing question is why free-living N_2 – fixing heterocystous cyanobacteria are not more abundant in N-deplete tropical open ocean where they would enjoy an obvious advantage over non-diazotrophic taxa? One explanation is that these heterocystous taxa are sensitive to periodic high turbulence (vertical wind mixing, storms) and the detrimental effects of smaller scale shear in these

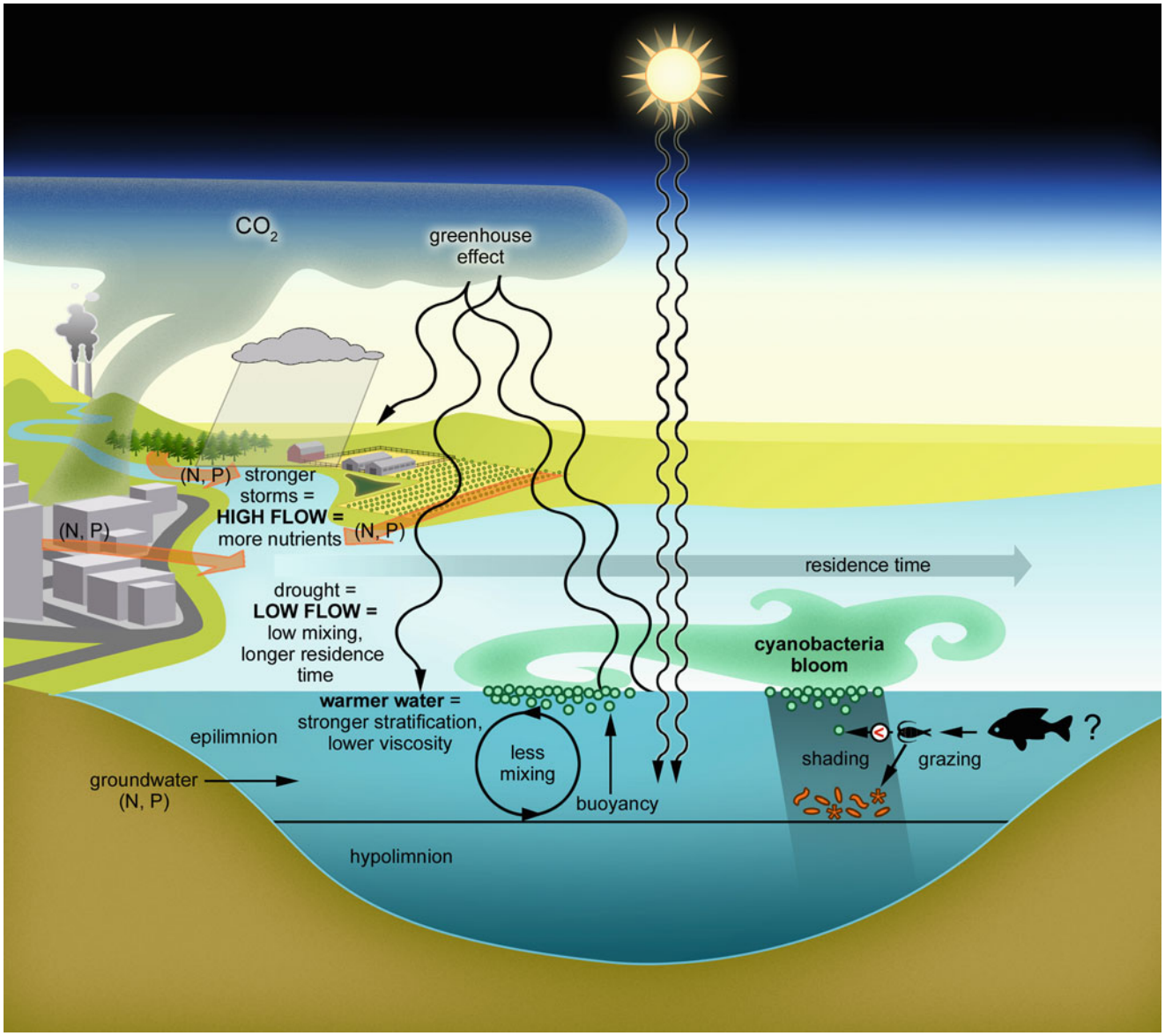

Fig. 5.10 Conceptual diagram, showing the direct and indirect effects of climate change on marine planktonic cyanobacterial growth and bloom dynamics. The impacts of regional and global warming attributed to enhanced greenhouse emissions are illustrated. Impacts include enhanced surface warming and stronger vertical stratification. Buoyant bloom-forming cyanobacterial taxa can take advantage of these changes, and, while dominating surface waters, they more effectively shade non-buoyant, deeper dwelling phytyoplankton populations. The effects of altered hydrologic conditions, including changes in water residence time brought on by more extreme rainfall as well as drought events, are also included

waters (Kucera 1996; Moisander et al. 2002b). Living endosymbiotically inside large diatoms (*Rhizosolenia* spp.) provides protection from these physical forcing features. An alternative hypothesis implicates a possible role of temperature in selecting for filamentous non-heterocystous cyanobacteria. Staal et al. (2003) showed that the diffusion of oxygen into N_2-fixing cells is constrained by the solubility of oxygen (which is a function of temperature and salinity) and that there was no advantage for a heterocyst cell wall in the tropical open ocean, since oxygen diffusion would limit the respiration required to support N_2 fixation. However, benthic heterocystous N_2 fixers are commonly observed under these conditions. Therefore, other factors, including turbulence, vertical mixing and other advective processes may play an interactive role in the control of this important group.

5.3.2.7 Light

Like eukaryotic phytoplankton genera, cyanobacteria have well-defined requirements for light to support photosynthetic and other metabolic activities (e.g. nitrogen fixation, nitrate assimilation, generation of energy-rich compound–ATP–used to support basic cell processes). Cyanobacterial light

requirements to sustain growth and reproduction are not dramatically different from those of eukaryotes. However, by adjusting their position in the water column through buoyancy compensation (Konopka 1984; Walsby 1992), cyanobacteria are particularly well-adapted to position themselves in an optimal light field. Even in persistent surface blooms, cyanobacteria are able to protect themselves from high irradiance and UV through the production of light shielding and absorbing (and hence protecting) compounds, including a range of carotenoids (Paerl et al. 1983; Paerl 1990) and scytonemins (Garcia-Pichel and Castenholz 1993). This photoprotective feature reflects the long evolutionary history of cyanobacteria, in which they experienced periods of very high UV irradiance during the early evolution of the Earth's atmosphere (Knoll 2003). Interestingly, marine bloom-forming cyanobacterial populations cultured in the laboratory (under artificial light) seem to lose their photoprotective capabilities (Paerl 1994).

5.3.2.8 Turbulence

Turbulence, over a range of scales, from micron-level shear to multi-meter vertical mixing, exerts a strong impact on phytoplankton growth and structural integrity (Fogg et al. 1973; Thomas and Gibson 1990). Increased levels of turbulence have been shown to inhibit growth of both non-diazotrophic and diazotrophic cyanobacteria (Fogg 1982; Fogg et al. 1973; Moisander et al. 2002b; Huisman et al. 2004). It follows that marine waters with persistent elevated turbulence may have a lower abundance of active N_2-fixing heterocystous cyanobacteria. In laboratory experiments where shear rates representative of surface wind-mixed conditions were applied to bloom-forming cyanobacteria (*Anabaena*, *Nodularia*), Kucera (1996) and Moisander et al. (2002b) showed that rates of N_2 fixation and photosynthesis could be suppressed by moderate to strong turbulence. However, other larger scale (larger than shear) studies did not find a negative relationship between turbulence and N_2 fixation (Howarth et al. 1993; Keto et al. 1992; Nakano et al. 2001, Burford 2006). The negative impacts of elevated shear could be due to: (1) breakage or weakening of cyanobacterial filaments at the delicate heterocyst-vegetative cell junction, causing O_2 inactivation of nitrogenase (Fogg 1969), and (2) disruption of bacterial-cyanobacterial associations (Paerl 1990).

It is suspected that shear and vertical mixing may have quite different, and possibly opposing, impacts on cyanobacterial activity, integrity and growth. In fact, low levels of mixing or swirling can stimulate the growth and biomass buildup of diverse phytoplankton groups (Stein 1973), including some cyanobacteria. Therefore, turbulence thresholds appear to exist, below which growth may be stimulated and above which it may be inhibited. Although they are difficult to measure and assess, the magnitudes, periodicity and spatial extent of various types and levels of turbulence and water motion over a range of scales appears to play fundamentally-important roles in controlling marine phytoplankton community composition, succession (Margalef 1968, 1978), and in the case of cyanobacterioplankton, determining the presence, persistence and dominance of bloom and non bloom-forming species (Reynolds 1987; Paerl 1988; Paerl et al. 2001). This may help explain why certain highly-turbulent estuarine systems that seem chemically-favourable for dominance by cyanobacterial bloom species (i.e. high nutrient loads and low N:P ratios; c.f. Smith 1983, 1990), are conspicuously devoid of these species (Paerl and Fulton 2006; Paerl et al. 1995a, b) (e.g. Chesapeake Bay, Neuse-Pamlico Sound, Florida Bay). However, in these systems, a significant fraction of the phytoplankton biomass can be cyanobacterial. Representative cyanobacteria tend to be small, single celled coccoid and thin filamentous types (e.g. *Synechococcus*, *Phormidium*) as opposed to larger filamentous and aggregated bloom species. Chemically-favourable (for cyanobacteria) estuarine, coastal and open ocean systems exhibiting strong seasonal persistent vertical stratification, can, at times support extensive and persistent cyanobacterial surface blooms of N_2 fixing and non-N_2 fixing species. Examples include the Baltic Sea, oceanic gyre systems (N. Pacific Gyre, Sargasso Sea) and physically "well-organized" (vertically and horizontally) current systems (e.g. Kurushio Current, Gulf Stream) (c.f. Paerl 1996; Capone et al. 1997).

Turbulence strongly interacts with nutrient supply, irradiance and temperature to determine the *potential* for cyanobacterial dominance and bloom formation (Paerl 1988; Reynolds 1987, 2006). If one physical variable strongly and consistently dominates, e.g., persistent cold temperatures, low light conditions, and high turbulence, cyanobacterial dominance (as bloom species) is generally precluded in nearshore and offshore waters.

An example of the high degree of sensitivity and response to varying degrees of turbulence is the periodic surface cyanobacterial blooms documented for the Baltic Sea. Such blooms typically form and persist in response to periods of water column stability, either as vertical stratification or along horizontal fronts, during summertime conditions when elevated temperatures and irradiance also favour cyanobacterial growth (Kononen 1992; Moisander et al. 1997; Sellner 1997; Kahru et al. 2000; Kononen et al. 1996; Kanoshina et al. 2003; Vahtera et al. 2005). Key indicators of cyanobacterial activity, such as rates of photosynthesis and N_2 fixation, showed similar positive responses to increased water column stability (reduced turbulence) (Moisander et al. 1997; Lehtimäki et al. 1997). When water column stability decreased, in response to fall cooling accompanied by increased vertical wind mixing, dominance by cyanobacterial bloom taxa gave way to dominance by eukaryotic bloom taxa, including dinoflagellates, cryptophytes and diatoms (Kononen et al. 1999). While cyanobacterial bloom taxa

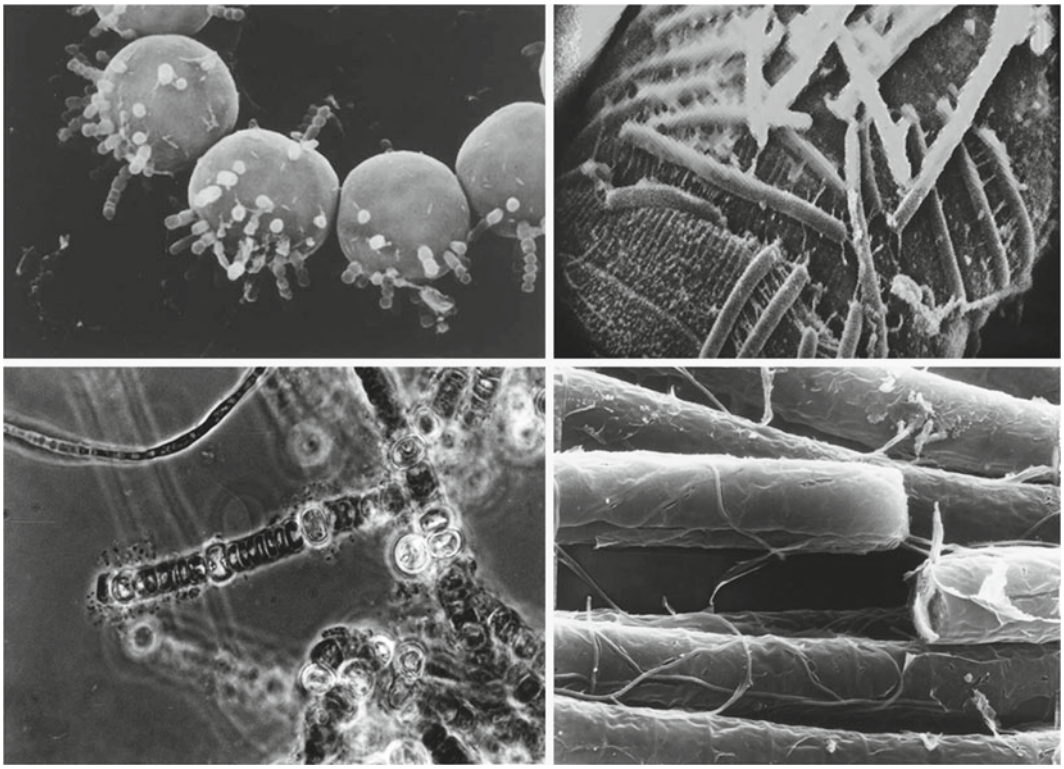

Fig. 5.11 Microbial associations with cyanobacteria freshly collected from various marine environments by the author. *Upper left* Scanning electron micrograph (*SEM*) of bacteria associated with vegetative cells of the filamentous heterocystous cyanobacteria *Nodularia* sp. during an active bloom (relatively high rates CO_2 and N_2 fixation were measured) in from the Baltic Sea. *Upper right* SEM of Bacteria closely associated with filaments of *Aphanizomenon* sp. in the brackish Chowan River, North Carolina. *Lower left* Phase contrast micrograph, showing bacteria aggregated around the heterocysts of *Nodularia* from a N_2-fixing bloom in the Baltic Sea. *Lower right* filamentous bacteria closely associated with filaments (trichomes) of *Trichodesmium*, sampled during a bloom in the Gulf Stream, Western Atlantic Ocean, off the coast of North Carolina

are negatively affected by increased turbulence, other cyanobacterial taxa (e.g. picocyanobacteria) are unaffected or may possibly even benefit. Kuosa (1991) showed that picocyanobacteria (<3 μm) are a widespread and important component of the Baltic Sea phytoplankton community (Kuparinen and Kuosa 1993; Albertano et al. 1997), especially during the warmer summer months. Diverse diazotrophs have also been identified within this picoplankton fraction (Farnelid et al. 2009).

5.3.2.9 Biotic Associations

Marine planktonic cyanobacteria form numerous obligate symbiotic and more mutualistic consortial relationships with other microorganisms and higher organisms. These include the symbiotic relationships between heterocystous cyanobacteria and the diatom genera *Rhizosolenia*, *Hemiaulus*, *Bacteriastrum* and *Chaetoceros* in the marine environment (Villareal 1992) (Fig. 5.4). Other symbioses have been reported (Carpenter and Foster 2002; Foster et al. 2006a, b; Escalera et al. 2010), including unicellular cyanobacterial associations with tintinnids, dinoflagellates and radiolarians (Foster et al. 2006a, b). Heterotrophic bacterial symbionts in the diatom genus *Rhizosolenia* have also been reported (Martinez et al. 1983). Evidence of N_2 fixation was found in symbionts of a dinoflagellate (*Histioneis* sp.) host using immunolabeling with nitrogenase coupled to TEM (Foster et al. 2006a). In addition, 16S rRNA sequences obtained from another *Histioneis* sp. host were similar to the diazotroph *Cyanothece* sp. ATCC, and provided evidence for N_2-fixing potential in the cyanobiont (Foster et al. 2006b).

Cyanobacterial-microbial associations are commonly observed among naturally occurring and cultured bloom-forming genera, including *Anabaena*, *Aphanizomenon*, *Nodularia*, and *Trichodesmium* (Paerl 1982, 2000; Paerl 1996). Examples of consortial N_2 fixation are the aggregates of the common heterocystous cyanobacterium *Nodularia* in the Baltic Sea, and species of the non-heterocystous aggregate forming *Lyngbya* as planktonic and benthic aggregates (Paerl and Kuparinen 2002). Associated microorganisms include eubacteria, fungi, protozoans and eukaryotic algae. Many associations appear intimate, occurring within and around colonies, aggregates of filaments and within the fibrillar-mucilaginous sheaths, capsules and exuded slimes (Paerl 1982) (Fig. 5.11).

Associations in which certain microbial populations exclusively attach to specific types of cyanobacterial host cells, i.e., akinetes and heterocysts, have also been observed (Fig. 5.11). Numerous investigations have shown that such specifically "bacterized" cyanobacterial species often exhibit higher cell-specific growth rates than axenic populations of the same species (Paerl 1982; Paerl and Pinckney 1996; Paerl and Kuparinen 2002). The mechanistic basis for cyanobacterial-bacterial synergism is poorly understood and remains the subject of current research. Proposed mutually-beneficial mechanisms include exchange of metabolites and growth factors as well as detoxifying roles of associated bacteria (Paerl 1982, 2000). On the other hand, such associations can also be antagonistic or symptomatic of intense competition for nutrients, light and other resources, and there is a vast literature illustrating such adverse relationships in planktonic habitats (Paerl 2000). Chemical communication, attraction and deterrence may also be involved in such associations. For example, Keating (1978) showed that substances excreted by cyanobacteria had growth-inhibiting effects on diatoms in the same phytoplankton community, while Herbst and Overbeck (1978) were able to demonstrate mutually-beneficial metabolic coupling between *Oscillatoria redekei* and accompanying bacteria. How and to what extent metabolites that are involved in cell to cell communication and quorum sensing establish and mediate these "metabolic relationships" are important informational needs (c.f. Axmann et al. 2005; Mearns-Spragg et al. 2005; Sharif et al. 2008). This information may yield key pieces of the puzzle clarifying the mechanistic underpinnings and regulation of cyanobacterial consortial and symbiotic associations in the marine environment, where ambient nutrient concentrations tend to be low and intracellular communication and nutrient exchange are likely to be quite important for optimizing growth and reproduction.

5.4 Marine Planktonic Cyanobacteria and Eutrophication

The accumulation of planktonic cyanobacterial blooms as bright green, yellow-brown or even red paint-like scum in estuarine and coastal waters is often symptomatic of nutrient over-enrichment and eutrophication (Fogg 1969; Paerl 1988; Paerl et al. 2001) (Fig. 5.12). Estuarine and coastal bloom-formers include members of the heterocystous genera

Fig. 5.12 Examples of marine cyanobacterial blooms. *Upper left*: A mixed *Anabaena* and *Microcystis* bloom in the brackish St. Johns River Estuary, Florida (Photo courtesy John Burns). *Upper right*: Open ocean (tropical Atlantic Ocean) *Trichodesmium* spp bloom (Photo courtesy Dr Ajit Subramanian Lamont Doherty Observatory, Columbia University, New York). *Lower left*: A mixed *Microcystis*, *Anabaenopsis*, *Anabeana* bloom in the brackish Chowan R.- Albemarle Sound, North Carolina (Photo H.W.P.). *Lower right*: Wind-blown *Nodularia* bloom, Baltic Sea (Photo courtesy Finnish Institute of Marine Research, Baltic Sea Portal)

Nodularia, *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, less frequently non-heterocystous genera *Lyngbya* and *Oscillatoria* and in brackish waters the non N_2 fixing genus *Microcystis* (Paerl 1988). Blooms can also be present in oligotrophic open ocean waters (e.g. *Trichodesmium*); however, such blooms are generally not indicative of eutrophication, but rather reflect physical conditions (calm, stratified waters) conducive to surface accumulations of this buoyant genus. Estuarine and coastal blooms can cause harm from ecological and health perspectives. Ecologically, blooms may be toxic to consumer species, causing food web alterations, with potentially detrimental effects on nutrient cycling, biodiversity and fisheries (Fogg 1969; Paerl et al. 2001). If they are not consumed, near-shore blooms can accumulate as massive amounts of organic matter, which when decomposed cause excessive oxygen consumption, hypoxia (<4 mg O_2 L^{-1}) or anoxia (no detectable O_2); major factors in the decline or elimination of fish, shellfish, invertebrate and plant habitats in coastal waters (Pihl et al. 1991; Rabalais and Turner 2001; Diaz and Rosenberg 2008). Moreover, hypoxic and anoxic sediments can release large amounts of nutrients (especially P and Fe) which may further stimulate phytoplankton growth and bloom potentials. In addition, N_2 fixing cyanobacterial blooms can constitute significant sources of "new" nitrogen (N), potentially impacting N-driven eutrophication and N cycling (Horne 1977; Paerl 1988); especially in semi-enclosed coastal seas and estuaries (e.g. Baltic Sea) where such blooms can be semi-permanent spring-fall features (Sellner 1997; Elmgren and Larsson 2001) (Figs. 5.5 and 5.12). Blooms can produce a variety of odor and taste compounds (geosmins, MIB), rendering affected waters unsuitable for consumption, aquaculture, swimming and other recreational activities (Carmichael 1997; Stewart and Falconer 2008). Lastly, numerous cyanobacterial bloom species produce alkaloids, peptides and other compounds that can be toxic upon ingestion or contact with affected waters (Carmichael 1997; Stewart and Falconer 2008).

At present, approximately 70% of the world's human population lives within 100 km of the coast, and this percentage continues to increase (Vitousek et al. 1997). Man's ever-increasing urban, agricultural and industrial encroachment on estuarine and coastal water- and airsheds has accelerated nutrient and other contaminant inputs, which have stimulated downstream/downwind primary production (eutrophication) and algal bloom potentials. This has also impacted biogeochemical cycling, food web dynamics and, from a fisheries perspective, sustainability on ecosystem, regional and global ocean scales.

To varying degrees, cyanobacterial blooms "track" cultural eutrophication in geographically-diverse nutrient-enriched waters, including, estuaries, embayments, brackish coastal and pelagic seas (i.e., Baltic) (Kononen 1992; Bianchi et al. 2000; Elmgren and Larsson 2001), lagoonal estuaries (Peele-Harvey, Australia), (Patos lagoon, Brazil) (Huber 1986; Yunes et al. 2004). The Baltic Sea has experienced the biotic and biogeochemical impacts of at least several centuries of cultural eutrophication (Elmgren 1989, 2001), in part manifested as cyanobacterial blooms (Finni et al. 2001). There is sedimentary evidence that indicates that cyanobacterial blooms have been present in this system for at least the past 100 years (Bianchi et al. 2000; Poutanen and Nikkilä 2001). Regional and global bloom expansion into more incipient eutrophying estuarine and coastal waters is underway however. Examples include the appearance, persistence and expansion of toxic (to wildlife, cattle, domestic animals and humans) non-heterocystous (*Microcystis*, *Lyngbya*) and heterocystous genera (*Anabaena, Anabaenopsis*, *Aphanizomenon, Nodularia*) in brackish fjords of Norway and Sweden, estuaries and coastal embayments of South Africa, Australia and New Zealand, Brazil, Columbia, Canada, Southeast Asia, and the USA (e.g. L. Ponchartrain, LA, Florida Bay, FL; Albemarle-Pamlico Sound System, NC), all under the influence of increasing urban, agricultural, and industrial loading of nutrients (Paerl 1997). Toxin and taste/odour-producing N_2 fixing taxa (*Anabaena, Anabaenopsis*, *Aphanizomenon*, *Lyngbya*, *Nodularia*) have become increasingly prevalent and problematic in aquaculture operations (Paerl and Tucker 1995; Carmichael 1997). Some of these genera (e.g. *Nodularia*) have species and strains capable invading and persisting in full-salinity and even hypersaline waters (Moisander et al. 2002a).

In addition to increasing nutrient loads and concentrations, ratios of the key limiting nutrients nitrogen and phosphorus (N:P) have been altered. Using an extensive data set from a range of freshwater lakes and reservoirs, Smith (1983) showed a strong relationship between total N:P ratios (by weight) and the prevalence of cyanobacterial bloom genera in freshwater environments. N:P ratios <20 were conducive to the development and periodic persistence of N_2-fixing genera. This stoichiometric predictor of cyanobacterial dominance has received surprisingly little attention and scrutiny in the marine environment (Smith 1990; Agawin et al. 2011), despite the fact that many estuarine and coastal waters exhibit N:P ratios well below 20, and are undergoing various symptoms and stages of cyanobacterial expansion (Niemi 1979; Paerl 1996; Paerl and Millie 1996).

N and P loads have increased so much in some receiving estuarine and coastal waters that stoichiometric predictions of either N_2-fixing or non-fixing bloom responses may not be meaningful; in part because bloom thresholds have been surpassed and factors other than nutrient supply control blooms (residence time, light availability, temperature, stratification). Examples include Scandinavian Fjords, Brazilian and Colombian coastal lagoons experiencing high nutrient loads from wastewater and aquaculture operations, the northern Gulf of Mexico region receiving nutrient laden discharge

from the Mississippi river, and the coastal South China Sea receiving discharge from the Yangtze River. In these cases, excessive anthropogenic N and/or P loading has been so high that nutrient limitations have shifted (from N to P to Si, and *vice versa*) (Elmgren and Larsson 2001; Sylvan et al. 2006; Conley et al. 2009a, b; Paerl 2009). Some of these perturbations have caused such large imbalances in nutrient input and concentration ratios, that the general assumption that freshwater systems are exclusively P limited and marine systems largely N limited has been called into question (Conley et al. 2009a, b; Paerl 2009).

However, in most N-sensitive estuarine and coastal waters experiencing N-driven eutrophication, the amounts and rates of N enrichment still do not exceed the ability of these waters to effectively assimilate this element. This is attributed to efficient phytoplankton N uptake kinetics that operate at sub-micromolar levels, thereby maintaining low ambient inorganic and organic N concentrations, and denitrification, the microbial conversion of nitrate to N_2 gas, which occurs at relatively high rates in estuarine and coastal shelf ecosystems (Seitzinger and Giblin 1996). Both processes remove biologically-available forms of N from the water column, especially in response to high N loading. Therefore, while N enrichment increases algal biomass and, hence, organic matter content (which is known to stimulate N_2 fixation), impacted waters generally exhibit N concentrations low enough to obviate either ammonium or nitrate inhibition of nitrogenase activity. As long as fertility is enhanced without parallel enhancement of ambient inorganic N concentrations, nitrogen limitation persists and conditions for N_2 fixation will continue to be favourable from a stoichiometric perspective. It is therefore curious, if not ironic, that cyanobacterial N_2 fixation is not more common and widespread in these waters. Clearly, factors in addition to nutrient supply and availability ratios play roles in controlling this process.

5.5 Managing Cyanobacteria in the Marine Environment

Identifying the suite of interactive environmental factors causing and sustaining harmful (toxin-producing, food web-altering, hypoxia-generating) cyanobacterial blooms (cyanoHABs) is a prerequisite to formulating controls of these manifestations of man-made nutrient over-enrichment and hydrologic modifications of coastal watersheds.

Because cyanobacterial bloom assemblages can have complex nutritional requirements, efforts aimed at controlling cyanoHAB dominance by manipulating only one nutrient (N or P) have met with mixed results. P input constraints are often most feasible and least costly. In some cases, P cutbacks can be highly effective on their own (without parallel N removal), because; (1) they may reduce total P availability enough to reduce growth of *all* bloom taxa, and (2) they may increase N:P ratios enough to provide eukaryotic algae a competitive advantage over cyanobacteria (Smith 1983, 1990; Downing et al. 2001; Schindler et al. 2008). In other cases, parallel N and P reductions have been needed to reduce bloom potentials (Elmgren and Larsson 2001; Paerl et al. 2004; Paerl 2009). In ecosystems where vast amounts of previously-loaded and/or naturally-occurring P reside in the sediments, both N and P reductions are required to reduce bloom potentials (Vollenweider and Kerekes 1982).

Many estuarine and coastal ecosystems fall into the latter category. These ecosystems are repositories of minerals derived from their watersheds and from exchange with the coastal ocean. Phosphorus compounds that enter these systems readily exchange between the water column and sediments, and over geological time scales (>1,000s of years) tend to accumulate in the sediments. This is because there are no significant gaseous forms of P; hence this element cannot escape from the system. In contrast, the N cycle contains multiple gaseous forms (e.g. NO, N_2O, NH_3, N_2). Therefore, fractions of the N pool can exchange with and escape into the atmosphere, while P is effectively retained in receiving marine systems. Processes controlling N exchange with the atmosphere include ammonification, denitrification, nitrous and nitric oxide production, products of the anammox reaction, and nitrogen fixation (Codispoti et al. 2001). Depending on the relative importance of influx vs. efflux reactions, N losses can outweigh gains in the marine environment. Assuming no human interference, the tendency is for N to assume the role as the most limiting nutrient (Ryther and Dunstan 1971; Nixon 1986).

It follows that N-enriched estuarine and coastal waters have experienced a recent upsurge in algal blooms (Paerl 1988; Hallegraeff 1993; Richardson 1997). Reducing N inputs has been recommended as a means of stemming coastal eutrophication (Vollenweider et al. 1992; Elmgren and Larsson 2001; Boesch et al. 2001). In the Neuse River Estuary, North Carolina, USA, deteriorating water quality has prompted calls for an N input cap and an overall 30% reduction in N loading (Paerl et al. 1995a, 2004). However, reductions in N loading alone may result in shifts in ratios of dissolved N to P supply rates in this and other N-sensitive estuaries (Paerl et al. 2004). Altered N:P inputs have significant impacts on aquatic communities far beyond a simple reduction in phytoplankton productivity and biomass, including shifts in species composition and possible selection for low N:P adapted species (Smith 1983; Tilman and Kiesling 1984). In particular, the phytoplankton community could become dominated by N_2 fixing cyanobacteria that may circumvent N-limitation imposed by N reductions. This appears to be the case in the Baltic Sea, as well as estuaries, coastal fjords and bays (Elmgren and Larsson 2001; Paerl et al. 2004; Paerl and Piehler 2008).

Both diazotrophic and non-diazotrophic cyanoHABs can utilize diverse forms of combined N and P (inorganic and organic) (Paerl 1988; Antia et al. 1991). This nutritional flexibility must be taken into consideration when predicting the potential cyanoHAB responses to anthropogenic N and P loadings. Non-N_2 fixing cyanoHAB genera, including salt-tolerant strains of *Microcystis*, *Lyngbya* and *Oscillatoria*, can exploit these N loading scenarios. Estuarine and coastal systems with relatively high externally-supplied P and N loads are attractive targets for these cyanoHABs. Examples include near-shore regions of the Baltic Sea, numerous Scandinavian Fjords, coastal regions and river deltas of the Mediterranean Sea, oligohaline regions of many anthropogenically-impacted estuaries (Danish estuaries, Brazilian lagoonal estuaries, Chesapeake Bay tributaries, North Carolina's Albemarle-Pamlico Sound system), and coastal lagoon systems draining urban and agricultural centers. In these systems, there is abundant evidence that *both* N and P reductions will be needed to reverse eutrophication and control cyanobacterial blooms (Elmgren and Larsson 2001; Paerl et al. 2004; Paerl 2009).

Overall, there is an emphasis on reducing N inputs to control estuarine and coastal eutrophication (c.f. Paerl 1997; Boesch et al. 2001; Elmgren and Larsson 2001). While this is undoubtedly a step in the right direction, the ramifications of reducing N relative to P in coastal waters with regard to cyanoHAB bloom potential needs to be carefully assessed. For example in the Neuse River Estuary, North Carolina, the potential for N_2 fixing cyanobacterial blooms (e.g. *Anabaena, Aphanizomenon*, and *Anabaenopsis*) exists (Piehler et al. 2002; Moisander et al. 2002a). This, combined with evidence that P loading also is excessive (Paerl 1987; Stow et al. 2001), suggests that if only N loading is reduced without parallel P reductions, the potential exists for replacing non-N_2 fixing *Microcystis* with N_2-fixing populations. Similarly, there is concern that reducing N without maintaining strict reductions on P inputs to control eutrophication in Sweden's Himmerfjärden, may allow cyanoHABs to regain dominance (Elmgren and Larsson 2001). Indeed, initial reductions of N in this fjord draining to the Baltic Sea have led to an increase in cyanobacterial biomass (Elmgren and Larsson 2001). Appropriately focused, timely monitoring of phytoplankton community structure responses to N reductions in these and other estuarine/coastal systems will enable managers to formulate N and P loading reductions effective in reversing eutrophication without promoting cyanoHABs.

5.6 Conclusions

Marine planktonic cyanobacteria are phylogenetically diverse and geographically widely distributed. Their activities, growth and proliferation as blooms are controlled by the interplay of a complex set of environmental variables, including physical (irradiance, temperature, turbulence), chemical (salinity, oxygen, organic matter, nitrogen, phosphorus and iron, as well trace metals), and biotic (grazing, consortial and symbiotic associations). Their long evolutionary history has led to an ability to tolerate and adapt to short-term (i.e., diel, seasonal, decadal) and longer term (geological) environmental changes, enabling these photosynthetic prokaryotes to be a "group for all seasons" in the salinity gradients representing the estuarine-coastal-open ocean continuum.

Despite their seemingly infinite adaptation to environmental change on both geological and biological time scales, cyanobacterial growth and bloom characteristics are specifically impacted by human and climatic alterations of aquatic environments. The most notable alteration is nutrient (especially N and P) enrichment or eutrophication. In addition, climatic factors such as warming, changes in frequencies and intensities of tropical cyclones, and protracted droughts, strongly affect growth potentials, geographic range and dominance of cyanobacteria in the world's oceans.

Effective management of CyanoHABs must address the above-mentioned suites of environmental factors, along with knowledge of the ecological and physiological adaptations that certain taxa possess to circumvent these controls. Examples include: (1) the ability of N_2-fixing taxa to exploit N-limited conditions, (2) the ability of certain buoyant taxa to counteract mixing and other means of man-induced destratification aimed at minimizing cyanobacterial dominance, (3) specific mutualistic and symbiotic associations with other microorganisms, higher plants and animals, which may provide clues as to the roles toxins and other chemical factors play in shaping biotic community structure and function.

Progress in identifying and understanding the roles toxins and other metabolites play in the physiology and ecology of bloom-forming cyanobacteria can be achieved by integrating physiological, toxicological and ecological perspectives and expertise. This includes hypothesis testing and problem solving using interdisciplinary (physical, chemical, biological/ecological) experimental, monitoring and assessment approaches. In addition, the synthesis of well defined laboratory experimental work with ecosystem-level studies utilizing similar techniques and measurements will prove invaluable in unraveling the complexity of environmental regulation of cyanobacterial bloom dynamics. Because of the expanding impacts human nutrient inputs, hydrologic, sediment and other perturbations are having on coastal regions and beyond, there is a need for more holistic, multi-prong approaches, including large-scale (ecosystem to regional) dual N and P reductions that include controls on atmospheric, surface runoff and subsurface discharge to the marine environment, for environmental problem solving.

The deployment of detection and identification techniques on appropriate spatial and temporal scales is a critically-important

component of any monitoring, assessment and modeling effort capable of characterizing cyanobacterial communities, their environmental controls and ecological/biogeochemical impacts in the marine environment. In this regard, significant and timely strides have been made in the fields of molecular biology and immunology, analytical chemistry, environmental sensing using electrochemical and optical techniques, remote sensing, and ecological modeling. Complimentary applications of these techniques will facilitate rapidly identify and quantify cyanobacterial populations, their potential biogeochemical and trophic roles, and their impacts on marine resources over a range of spatial scales (from meters to 100s of kilometers) and temporal scales (minutes to decades). This will enable researchers and managers to assess community/population status and trends in relation to anthropogenic and climatic drivers in order to quantitatively link environmental to biotic change in marine planktonic ecosystems.

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References

Agawin NSR, Duarte CM, Agusti S, Vaque D (2011) Effect of N:P ratios on response of Mediterranean picophytoplankton to experimental nutrient inputs. Aquat Microb Ecol 34:57–67

Albertano P, Di Somma D, Capucci E (1997) Cyanobacterial picoplankton from the Central Baltic Sea: cell size classification by image analysis fluorescence microscopy. J Plankton Res 19:1405–1416

Allen MM, Stanier RY (1968) Selective isolation of blue-green algae from water and soil. J Gen Microbiol 51:203–209

Antia NJ, Harrison PJ, Oliveira L (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1–89

Axmann IM, Kensche P, Vogel J, Kohl S, Herzel H, Hess WR (2005) Identification of cyanobacterial non-coding RNAs by comparative genome analysis. Genome Biol 6:R73

Berman-Frank I, Lundgren P, Chen YB, Kupper H, Kolber Z, Bergman B, Falkowski P (2001) Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*. Science 294:1534–1537

Bianchi TS, Engelhaupt E, Westman P, Andren T, Rolff A, Elmgren R (2000) Cyanobacterial blooms in the Baltic Sea: natural or human-induced? Limnol Oceanogr 48:716–726

Bishop PE, Premakumar R (1992) Alternative nitrogen fixation systems. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation, 1st edn. Routledge/Chapman and Hall, Inc., New York, pp 736–762

Boesch DF, Burreson E, Dennison W, Houde E, Kemp M, Kennedy V, Newell R, Paynter K, Orth R, Ulanowicz R (2001) Factors in the decline of coastal ecosystems. Science 293:629–638

Boesch DF, Hecky RE, O'Melia C, Schindler DW (2005) Eutrophication of Swedish Seas. Report 5509, MARS 2006, Swedish Environmental Protection Agency, Stockholm, Sweden

Bothe H, Tripp HJ, Zehr JP (2011) Unicellular cyanobacteria with a new mode of life: the lack of photosynthetic oxygen evolution allows nitrogen fixation to proceed. Arch Microbiol 192(10):783–790

Bou Karam D, Flamant C, Tulet P, Todd MC, Pelon J, Williams E (2009) Dry cyclogenesis and dust mobilization in the Inter Tropical Discontinuity of the West African Monsoon: a case study. J Geophys Res 114:D13106. doi:10.1029/2008JD011444

Boyd PW, Jickells T, Law CS, Blain S, Boyle EA, Bueseler KO, Coale KH, Culen JJ, de Baar HJW, Follows M, Harvey M, Lancelot C, Levasseur M, Owens NPJ, Pollard R, Rivkin RB, Sarmiento J, Schoemann V, Smetacek V, Takeda S, Tsuda A, Turner S, Watson AJ (2007) Mesoscale iron enrichment experiments 1993–2005: synthesis and future directions. Science 315:612–617

Burford MA (2006) A comparison of phytoplankton community assemblages in artificially and naturally mixed subtropical water reservoirs. Freshw Biol 51:973–982

Butterwick C, Heaney SI, Talling JF (2005) Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. Freshw Biol 50:291–300

Capone DG (1983) Benthic nitrogen fixation. In: Carpenter EJ, Capone DG (eds) Nitrogen in the marine environment. Academic, New York, pp 105–137, 900 pp

Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter EJ (1997) *Trichodesmium*, a globally-significant marine cyanobacterium. Science 276:1221–1229

Capone DG, Mulholland M, Carpenter EJ (eds) (2008) Nitrogen in the marine environment, vol 2. Academic, Orlando

Carmichael WW (1997) The cyanotoxins. Adv Bot Res 27:211–256

Carpenter EJ (2002) Marine cyanobacterial symbioses. Biol Environ Proc R Ir Acad 102B:15–18

Carpenter EJ, Capone DG (1992) Nitrogen fixation in *Trichodesmium* blooms. In: Carpenter DG, Capone EJ, Rueter JG (eds) Marine pelagic cyanobacteria: *Trichodesmium* and other Diazotrophs. Kluwer Academic Publishers, Dordrecht, pp 211–217, 357 pp

Carpenter EJ, Foster RA (2002) Marine symbioses. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 11–17, 355 pp

Carpenter EJ, Janson S (2001) *Anabaena gerdii* sp nov., a new planktonic filamentous cyanobacterium from the South Pacific Ocean and Arabian Sea. Phycologia 40:105–110

Carpenter EJ, Price CC IV (1976) Marine *Oscillatoria* (*Trichodesmium*): an explanation for aerobic nitrogen fixation without heterocysts. Science 191:1278–1280

Chen CT, Millero FJ (1986) Precise thermodynamic properties for natural waters covering only the limnological range. Limnol Oceanogr 31:657–662

Church MJ, Jenkins BD, Karl DM, Zehr JP (2005) Vertical distributions of nitrogen-fixing phylotypes at Station ALOHA in the oligotrophic North Pacific Ocean. Aquat Microb Ecol 38:3–14

Church MJ, Ducklow HW, Letelier RM, Karl DM (2006) Temporal dynamics in heterotrophic picoplankton productivity in the subtropical North Pacific Ocean. Aquat Microb Ecol 45:41–53

Church MJ, Björkman KM, Karl DM, Saito MA, Zehr JP (2008) Regional distributions of nitrogen fixing bacteria in the Pacific Ocean. Limnol Oceanogr 53:63–77

Codispoti LA, Brandes JA, Christensen JP, Devol AH, Naqvi SWA, Paerl HW, Yoshinari T (2001) The oceanic fixed nitrogen and nitrous oxide budgets: moving targets as we enter the anthropocene? Sci Mar 65(Supp. 2):85–105

Cole JJ, Lane JM, Marino R, Howarth RW (1993) Molybdenum assimilation by cyanobacteria and phytoplankton in freshwater and saltwater. Limnol Oceanogr 38:25–35

Conley DJ, Carstensen J, Vaquer-Sunyer R, Duarte CM (2009a) Ecosystems thresholds with hypoxia. Hydrobiologia 629:21–29
Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009b) Controlling eutrophication: nitrogen and phosphorus. Science 323:1014–1015
D'Elia CF, Sanders JG, Boynton WR (1986) Nutrient enrichment studies in a coastal plain estuary: phytoplankton growth in large scale, continuous cultures. Can J Fish Aquat Sci 43:397–406
De Senerpont Domis LN, Mooij WM, Huisman J (2007) Climate-induced shifts in an experimental phytoplankton community: a mechanistic approach. Hydrobiologia 584:403–413
Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321:926–929
Dortch Q, Parsons ML, Rabalais NN, Turner RE (1999) What is the threat of harmful algal blooms in Louisiana Coastal Waters? In: Rozas LP, Nyman JA, Proffitt CE, Rabalais NN, Reed DJ, Turner RE (eds) Recent research in Coastal Louisiana: natural system function and response to human influences. Louisiana Sea Grant College Program, Baton Rouge
Downing JA, Watson SB, McCauley E (2001) Predicting cyanobacteria dominance in lakes. Can J Fish Aquat Sci 58:1905–1908
Du Bois JD, Kapusta LA (1981) Osmotic stress effects on the $N_2(C_2H_2ASA)$ activity of aquatic cyanobacteria. Aquat Bot 11:11–20
Dugdale RC (1967) Nutrient limitation in the sea: dynamics, identification and significance. Limnol Oceanogr 12:685–695
El-Shehawy R, Lugomela C, Ernst A, Bergman B (2003) Diurnal expression of *hetR* and diazocyte development in the filamentous non-heterocystous cyanobacterium *Trichodesmium erythraeum*. Microbiology 149:1139–1146
Elliott JA, Jones ID, Tackeray SJ (2006) Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. Hydrobiologia 559:401–411
Elmgren R (1989) Man's impact on the ecosystem of the Baltic Sea; energy flows today and at the turn of the century. Ambio 18:326–332
Elmgren R (2001) Understanding human impact on the Baltic ecosystem: changing views in recent decades. Ambio 30:222–231
Elmgren R, Larsson U (2001) Nitrogen and the Baltic Sea: managing nitrogen in relation to phosphorus. Sci World 1(S2):371–377
Escalera L, Reguera B, Takishita K, Yoshimatsu Y, Koike K, Koike K (2010) Cyanobacterial endosymbionts in the benthic dinoflagellate *Sinophysis canaliculata* (Dinophysiales, Dinophyceae). Protist. doi:10.1016/j.protis.2010.07.003
Falcón LI, Carpenter EJ, Cipriano F, Bergman B, Capone DG (2004) N_2 fixation by unicellular bacterioplankton from the Atlantic and Pacific Oceans: phylogeny and *in situ* rates. Appl Environ Microbiol 70:765–770
Farnelid H, Öberg T, Riemann L (2009) Identity and dynamics of putative N_2-fixing picoplankton in the Baltic Sea proper suggest complex patterns of regulation. Environ Microbiol Rep 1:145–154
Finni T, Kononen K, Olsonen R, Wallström K (2001) The history of cyanobacterial blooms in the Baltic Sea. Ambio 30:172–178
Fisher TR, Peele ER, Ammerman JA, Harding LW (1992) Nutrient limitation of phytoplankton in Chesapeake Bay. Mar Ecol Prog Ser 82:51–63
Fogg GE (1956) The comparative physiology and biochemistry of the blue-green algae. Bacteriol Rev 20:148–165
Fogg GE (1969) The physiology of an algal nuisance. Proc R Soc Lond Ser B 173:175–189
Fogg GE (1982) Marine plankton. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell/University of California Press, Oxford/Berkeley, pp 491–513, 688 pp
Fogg GE, Stewart WDP, Fay P, Walsby AE (1973) The blue-green algae. Academic, London
Fong AA, Karl DM, Lukas R, Letelier RM, Zehr JP, Church MJ (2008) Nitrogen fixation in an anticyclonic eddy in the oligotrophic North Pacific Ocean. ISME J 2:663–676
Foster RA, O'Mullan GD (2008) Nitrogen-fixing and nitrifying symbioses in the marine environment. In: Capone DG, Bronk D, Mulholland M, Carpenter EJ (eds) Nitrogen in the marine environment, 2nd edn. Elsevier Science, Amsterdam, pp 1197–1218
Foster RA, Zehr JP (2006) Characterization of diatom-cyanobacteria symbioses on the basis of *nifH*, *hetR*, and 16S rRNA sequences. Environ Microbiol 8:1913–1925
Foster RA, Bergman B, Carpenter EJ (2006a) Unicellular cyanobionts in open ocean dinoflagellates, radiolarians, and tintinnids: ultrastructural characterization and immuno-localization of phycoerythrin and nitrogenase. J Phycol 42:453–463
Foster RA, Collier JA, Carpenter EJ (2006b) Reverse transcription PCR amplification of cyanobacterial symbiont 16S rRNA sequences from single non-photosynthetic eukaryotic marine planktonic host cells. J Phycol 42:243–250
Foster RA, Subramaniam A, Zehr JP (2008) Distribution and activity of diazotrophy in the Eastern Equatorial Atlantic. Environ Microbiol 11:741–750
Foy RH, Gibson CE, Smith RV (1976) The influence of daylength, light intensity and temperature on the growth rates of planktonic blue-green algae. Eur J Phycol 11:151–163
Fredriksson C, Bergman B (1995) Nitrogenase quantity varies diurnally in a subset of cells within colonies of the non-heterocystous cyanobacteria *Trichodesmium* spp. Microbiology 141:2471–2478
Gallon JR (1992) Tansley review no. 44/reconciling the incompatible: N_2 fixation and O_2. New Phytol 122:571–609
Garcia-Pichel F, Castenholz RW (1993) Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl Environ Microbiol 59:163–169
Gómez F, Furuya K, Takeda S (2005) Distribution of the cyanobacterium *Richelia intracellularis* as an epiphyte of the diatom *Chaetoceros compressus* in the western Pacific Ocean. J Plankton Res 27:323–330
Hallegraeff GM (1993) A review of harmful algal blooms and their apparent global increase. Phycologia 32:79–99
Herbst V, Overbeck J (1978) Metabolic coupling between the alga *Oscillatoria redekei* and accompanying bacteria. Naturwissenschaften 65:598–599
Holland HD (1978) The chemistry of the atmosphere and oceans. Wiley, New York, 341 pp
Holm-Hansen O (1964) Physiology of algae. In: Encyclopedia of science and technology, yearbook. McGraw-Hill, Inc., New York, pp 129–132
Hood RR, Bates NR, Capone DG, Olson DB (2001) Modeling the effect of nitrogen fixation on carbon and nitrogen fluxes at BATS. Deep Sea Res 48:1609–1648
Hood RR, Subramanian A, May LR, Carpenter EJ, Capone DG (2002) Remote estimation of nitrogen fixation by *Trichodesmium*. Deep Sea Res 49:123–147
Hood RR, Coles VJ, Capone DG (2004) Modeling the distribution of *Trichodesmium* and nitrogen fixation in the Atlantic Ocean. J Geophys Res Ocean 109:1–25
Horne AJ (1977) Nitrogen fixation.- a review of this phenomenon as a polluting process. Prog Water Technol 8:359–372
Howarth RW, Cole JJ (1985) Molybdenum availability, nitrogen availability, and phytoplankton growth in natural waters. Science 229:653–655
Howarth RW, Butler T, Lunde K, Swaney D, Chu CR (1993) Turbulence and planktonic nitrogen fixation: a mesocosm experiment. Limnol Oceanogr 38:1696–1711
Huber AL (1986) Nitrogen fixation by *Nodularia spumigena* Martens (Cyanobacteria). I. Field studies on the contribution of blooms to the nitrogen budget of the Peel-Harvey Estuary, Western Australia. Hydrobiologia 131:193–203
Huisman J, Sharples J, Stroom J, Visser PM, Kardinaal WEA, Verspagen JMH, Sommeijer B (2004) Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology 85:2960–2970

Huisman JM, Matthijs HCP, Visser PM (2005) Harmful cyanobacteria, Springer aquatic ecology series 3. Springer, Dordrecht

Janson S, Carpenter EJ, Bergman B (1993) Compartmentalisation of nitrogenase in a non-heterocystous cyanobacterium: *Trichodesmium contortum*. FEMS Microbiol Lett 118:9–14

Janson S, Wouters J, Bergman B, Carpenter EJ (1999) Host specificity in the *Richelia* diatom symbiosis revealed by hetR gene sequence analysis. Environ Microbiol 1:431–438

Jöhnk KD, Huisman J, Sharples J, Sommeijer B, Visser PM, Stroom JM (2008) Summer heatwaves promote blooms of harmful cyanobacteria. Glob Change Biol 14:495–512

Johnson PW, Sieburth JM (1979) Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. Limnol Oceanogr 24:928–935

Kahru M, Leppänen JM, Rud O (1993) Cyanobacterial blooms cause heating of the sea surface. Mar Ecol Prog Ser 101:1–7

Kahru M, Leppänen JM, Rud O, Savchuk OP (2000) Cyanobacteria blooms in the Gulf of Finland triggered by saltwater inflow into the Baltic Sea. Mar Ecol Prog Ser 207:13–18

Kana TM (1993) Rapid oxygen cycling in *Trichodesmium thiebautii*. Limnol Oceanogr 38:18–24

Kanoshina I, Lips U, Leppänen JM (2003) The influence of weather conditions (temperature and wind) on cyanobacterial bloom development in the Gulf of Finland (Baltic Sea). Harmful Algae 2:29–41

Karl D, Michaels A, Bergman B, Capone D, Carpenter E, Letelier R, Lipschultz F, Paerl H, Sigman D, Stal L (2002) Dinitrogen fixation in the world's oceans. Biogeochemistry 57(58): 47–98

Keating KI (1978) Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure. Science 199:971–973

Keto J, Horppila J, Kairesalo T (1992) Regulation of the development and species dominance of summer phytoplankton in Lake Vesijarvi – predictability of enclosure experiments. Hydrobiologia 243:303–310

Kirrikki M, Inkala A, Kuosa H, Pitkänen H, Kuussisto M, Sarkuula J (2001) Evaluating the effects of nutrient load reductions on the biomass of toxic nitrogen-fixing cyanobacteria in the Gulf of Finland, Baltic Sea. Boreal Environ Res 6:131–146

Knoll A (2003) Life on a young planet: the first three billion years of evolution on earth. Princeton University Press, Princeton

Komarék J, Anagnostidis K (1986) Modern approach to the classification system of cyanophytes. Archiv Hydrobiol Suppl 73 Algol Stud 43:157–164

Kononen K (1992) Dynamics of the toxic cyanobacterial blooms in the Baltic Sea. Finn Mar Res 261:3–36

Kononen K, Kuparinen J, Mäkelä J, Laanemets J, Pavelson J, Nõmmann S (1996) Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea. Limnol Oceanogr 41:98–112

Kononen K, Huttunen M, Kanoshina I, Laanemets J, Moisander P, Pavelson J (1999) Spatial and temporal variability of a dinoflagellate-cyanobacterium community under a complex hydrodynamical influence: a case study at the entrance to the Gulf of Finland. Mar Ecol Prog Ser 186:43–57

Konopka A (1984) Effect of light-nutrient interactions on buoyancy regulation by planktonic cyanobacteria. In: Klug MJ, Reddy CA (eds) Current perspectives in microbial ecology. American Society for Microbiology, Washington, DC, pp 41–48

Konopka A, Brock TD, Walsby AE (1978) Buoyancy regulation by planktonic blue-green algae in Lake Mendota, Wisconsin. Arch Hydrobiol 83:524–537

Kucera SA (1996) The influence of small-scale turbulence on N_2 fixation and growth in heterocystous cyanobacteria. M. S. thesis, University of North Carolina, Chapel Hill

Kuosa H (1991) Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing. Mar Ecol Prog Ser 73:269–276

Kuparinen J, Kuosa H (1993) Autotrophic and heterotrophic picoplankton in the Baltic Sea. Adv Mar Biol 29:73–128

Küpper H, Ferimazova N, Setlik I, Berman-Frank I (2004) Traffic lights in *Trichodesmium*. Regulation of photosynthesis for nitrogen fixation studied by chlorophyll fluorescence kinetic microscopy. Plant Physiol 135:2120–2133

Lange W (1967) Effects of carbohydrates on these symbiotic growth of the planktonic blue-green algae with bacteria. Nature 215:1277–1278

Lehtimäki J, Moisander P, Sivonen K, Kononen K (1997) Growth, nitrogen fixation and nodularin production by two Baltic Sea Cyanobacteria. Appl Environ Microbiol 63:1647–1656

Lenes JM, Walsh JJ, Otis DB, Carder KL (2005) Iron fertilization of *Trichodesmium* off the west coast of Barbados: a one-dimensional numerical model. Deep Sea Res 52:1021–1041

Leredulier DT, Bernard T, Goas G, Hamelin J (1984) Osmoregulation in *Klebsiella pneumoniae*: enhancement of anaerobic growth and nitrogen fixation under stress by proline betaine, γ-butyrobetaine, and other related compounds. Can J Microbiol 30:299–305

Likens GE (ed) (1972) Nutrients and eutrophication, American Society of Limnology Oceanography special symposium 1. American Society of Limnology Oceanography, Lawrence

Margalef R (1968) Perspectives in ecological theory. University of Chicago Press, Chicago

Margalef R (1978) Life-forms of phytoplankton as survival alternatives in an unstable environment. Oceanol Acta 1:493–509

Marshall HG (2002) Autotrophic picoplankton: their presence and significance in marine and freshwater ecosystems. Va J Sci 53:13–33

Martin JH et al (1994) Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature 371:123–129

Martinez L, Silver MW, King JM, Alldredge AL (1983) Nitrogen fixation by floating diatom mats: a source of new nitrogen to oligotrophic ocean waters. Science 221:152–154

Mearns-Spragg M, Bregu K, Boyd G, Burgess JG (2005) Cross-species induction and enhancement of antimicrobial activity produced by epibiotic bacteria from marine algae and invertebrates, after exposure to terrestrial bacteria. Lett Appl Microbiol 27:142–146

Moisander PH, Paerl HW (2000) Growth, primary productivity, and nitrogen fixation potential of *Nodularia* spp. (Cyanophyceae) in water from a subtropical estuary in the United States. J Phycol 36:645–658

Moisander PH, Rantajärvi E, Huttunen M, Kononen K (1997) Phytoplankton community in relation to salinity fronts at the entrance to the Gulf of Finland, Baltic Sea. Ophelia 46:187–203

Moisander PH, Hench JL, Kononen K, Paerl HW (2002a) Small-scale shear effects on heterocystous cyanobacteria. Limnol Oceanogr 47:108–119

Moisander PH, McClinton E III, Paerl HW (2002b) Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microb Ecol 43:432–442

Moisander PH, Breinart RA, Hewson I, White AS, Johnson KS, Carolson CA, Montoya JP, Zehr JP (2010) Unicellular cyanobacterial distributions broaden the oceanic N_2 fixation domain. Science 327:1512–1514

Murphy TO, Lean DRS, Nalewajko C (1976) Blue-green algae: their excretion of selective chelators enables them to dominate other algae. Science 221:152–154

Murrell MC, Lores EM (2004) Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria. J Plankton Res 26:371–382

Nakano S, Hayakawa K, Frenette JJ, Nakajima T, Jiao CM, Tsujimura S, Kumagai M (2001) Cyanobacterial blooms in a shallow lake: a large-scale enclosure assay to test the importance of diurnal stratification. Arch Hydrobiol 150:491–509

Nausch G, Nehring D, Aertebjerg G (1999) Anthropogenic nutrient load of the Baltic Sea. Limnologica 29:233–241
Neilands JB (1967) Hydroxamic acids in nature. Science 156:1443–1447
Niemi A (1979) Blue-green algal blooms and N:P ratio in the Baltic Sea. Acta Bot Fenn 110:57–61
Nixon SW (1986) Nutrient dynamics and the productivity of marine coastal waters. In: Halwagy R, Clayton D, Behbehani M (eds) Coastal eutrotrophication. The Alden Press, Oxford, pp 97–115
Nixon SW (1995) Coastal eutrophication: a definition, social causes, and future concerns. Ophelia 41:199–220
Paerl HW (l982) Interactions with bacteria. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 441–461, 688 pp
Paerl HW (1983) Environmental factors promoting and regulating N_2 fixing blue-green algal blooms in the Chowan River, NC. University of North Carolina Water Resources Research Institute report no. 176. Water Resources Research Institute of the University of North Carolina, Raleigh, 65 pp
Paerl HW (1986) Growth and reproductive strategies of freshwater blue-green algae (cyanobacteria). In: Sandgren CD (ed) Growth and reproductive strategies of freshwater Phytoplankton. Cambridge University Press, Cambridge
Paerl HW (1987) Dynamics of blue-green algal blooms in the lower Neuse River, NC: causative factors and potential controls. University of North Carolina Water Resources Research Institute report no 229. Water Resources Research Institute of the University of North Carolina, Raleigh, 164 pp
Paerl HW (1988) Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol Oceanogr 33:823–847
Paerl HW (1990) Physiological ecology and regulation of N_2 fixation in natural waters. Adv Microbiol Ecol 11:305–344
Paerl HW (1994) Spatial segregation of CO_2 fixation in *Trichodesmium* sp.: linkage to N_2 fixation potential. J Phycol 30:790–799
Paerl HW (1996) A comparison of cyanobacterial bloom dynamics is freshwater, estuarine and marine environments. Phycologia 35(6):25–35
Paerl HW (1997) Coastal eutrophication and harmful algal blooms: importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnol Oceanogr 42:1154–1165
Paerl HW (1999) Physical-chemical constraints on cyanobacterial growth in the oceans. In: Charpy L, Larkum AWD (eds) Marine cyanobacteria, Bulletin de l'Institut océanographique, Monaco. Special vol no. 19. Musée océanographique, Monaco, pp 319–349
Paerl HW (2000) Marine plankton. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Scientific Publications, Dordrecht, pp 121–148, 669 pp
Paerl HW (2008) Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum. Adv Exp Med Biol 619:216–241
Paerl HW (2009) Controlling eutrophication along the freshwater–marine continuum: dual nutrient (N and P) reductions are essential. Estuar Coasts 32:593–601
Paerl HW, Bebout BM (1988) Direct measurement of O_2-depleted microzones in marine *Oscillatoria*: relation to N_2 fixation. Science 242:441–445
Paerl HW, Fulton RS (2006) Ecology of harmful cyanobacteria. In: Graneli E, Turner J (eds) Ecology of harmful marine algae. Springer, Berlin, pp 95–107
Paerl HW, Huisman J (2008) Blooms like it hot. Science 320:57–58
Paerl HW, Huisman J (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ Microbiol Rep 1(1):27–37
Paerl HW, Kuparinen J (2002) Microbial aggregates and consortia. In: Bitton G (ed) Encyclopedia of environmental microbiology, vol 1. Wiley, New York, pp 160–181
Paerl HW, Millie DF (1996) Physiological ecology of toxic cyanobacteria. Phycologia 35:160–167
Paerl HW, Piehler MF (2008) Nitrogen and marine eutrophication. In: Capone DG, Mulholland M, Carpenter E (eds) Nitrogen in the marine environment, vol 2. Academic, Orlando, pp 529–567
Paerl HW, Pinckney JL (1996) Microbial consortia: their role in aquatic production and biogeochemical cycling. Microb Ecol 31:225–247
Paerl HW, Tucker C (1995) Ecology of blue-green algae in aquaculture ponds. J World Aquacult Soc 26:1–53
Paerl HW, Whitall DR (1999) Anthropogenically-derived atmospheric nitrogen deposition, marine eutrophication and harmful algal bloom expansion: is there a link? Ambio 28:307–311
Paerl HW, Zehr JP (2000) Nitrogen fixation. In: Kirchman DJ (ed) Microbial ecology of the oceans. Academic, New York, pp 387–426, 542 pp
Paerl HW, Tucker J, Bland PT (1983) Carotenoid enhancement and its role in maintaining blue-green algal (*Microcystis aeruginosa*) surface blooms. Limnol Oceanogr 8:847–857
Paerl HW, Priscu JC, Brawner DL (1989) Immunochemical localization of nitrogenase in marine *Trichodesmium* aggregates: relationship to N_2 fixation potential. Appl Environ Microbiol 55:2965–2975
Paerl HW, Prufert LE, Ambrose WW (1991) Contemporaneous N_2 fixation and oxygenic photosynthesis in the non-heterocystous mat-forming cyanobacterium *Lyngbya aestuarii*. Appl Environ Microbiol 57:3086–3092
Paerl HW, Prufert-Bebout L, Guo C (1994) Iron-stimulated N_2 fixation and growth in natural and cultured populations of the planktonic marine cyanobacterium *Trichodesmium*. Appl Environ Microbiol 60:1044–1047
Paerl HW, Mallin MA, Donahue CA, Go M, Peierls BL (1995a) Nitrogen loading sources and eutrophication of the Neuse River Estuary, NC: direct and indirect roles of atmospheric deposition. University of North Carolina Water Resources Research Institute report no 291. Water Resources Research Institute of the University of North Carolina, Raleigh
Paerl HW, Pinckney JL, Kucera S (1995b) Clarification of the structural and functional roles of heterocysts and anoxic microzones in the control of pelagic nitrogen fixation. Limnol Oceanogr 40:634–638
Paerl HW, Willey JD, Go M, Peierls BL, Pinckney JL, Fogel ML (1999) Rainfall stimulation of primary production in Western Atlantic Ocean waters: roles of different nitrogen sources and co-limiting nutrients. Mar Ecol Prog Ser 176:205–214
Paerl HW, Fulton RS, Moisander PH, Dyble J (2001) Harmful freshwater algal blooms, with an emphasis on cyanobacteria. Sci World 1:76–113
Paerl HW, Valdes LM, Piehler MF, Lebo ME (2004) Solving problems resulting from solutions: The evolution of a dual nutrient management strategy for the eutrophying Neuse River Estuary, North Carolina, USA. Environ Sci Technol 38:3068–3073
Paerl HW, Hall NS, Calandrino ES (2011a) Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. Sci Total Environ 409:1739–1745
Paerl RW, Johnson KS, Welsh RM, Worden AZ, Chavez FP, Zehr JP (2011b) Differential distributions of *Synechococcus* subgroups across the California current system. Front Microbiol 2:59. doi:10.3389/fmicb.2011.0005
Panosso R, Granéli E (2000) Effects of dissolved organic matter on the growth of *Nodularia spumigena* (Cyanophyceae) cultivated under N and P deficiency. Mar Biol 136:331–336
Paulsen DM, Paerl HW, Bishop PE (1991) Evidence that molybdenum-dependent nitrogen fixation is not limited by high sulfate in marine environments. Limnol Oceanogr 36:1325–1334
Pearsall W (1932) Phytoplankton in the English Lakes. 2. The composition of the phytoplankton in relation to dissolved substances. J Ecol 20:241–262
Peeters F, Straile D, Lorke A, Livingstone DM (2007) Earlier onset of the spring phytoplankton bloom in lakes of the temperate zone in a warmer climate. Glob Change Biol 13:1898–1909

Phlips EJ, Badylak S, Lynch TC (1999) Blooms of the picoplanktonic cyanobacterium *Synechococcus* in Florida Bay, a subtropical inner-shelf lagoon. Limnol Oceanogr 44:1166–1175

Piehler MF, Dyble J, Moisander PH, Pinckney JL, Paerl HW (2002) Effects of modified nutrient concentrations and ratios on the structure and function of the native phytoplankton community in the Neuse River Estuary, North Carolina USA. Aquat Ecol 36:371–385

Pihl L, Baden SP, Diaz RJ (1991) Effects of periodic hypoxia on distributions of demersal fish and crustaceans. Mar Biol 108:349–360

Pinckney JL, Millie DF, Vinyard BT, Paerl HW (1997) Environmental controls of phytoplankton bloom dynamics in the Neuse River Estuary (North Carolina, USA). Can J Fish Aquat Sci 54:2491–2501

Potts M (1980) Blue-green algae (cyanophyta) in marine coastal environment of the Sinai Peninsula: distribution, zonation, stratification and taxonomic diversity. Phycologia 19:60–73

Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755–805

Poutanen EL, Nikkilä K (2001) Carotenoid pigments as tracers of cyanobacterial blooms in recent and post-glacial sediments of the Baltic Sea. Ambio 30:179–183

Prufert-Bebout L, Paerl HW, Lassen C (1993) Growth, nitrogen fixation, and spectral attenuation in cultivated *Trichodesmium* species. Appl Environ Microbiol 59:1367–1375

Rabalais NN, Turner RE (eds) (2001) Coastal hypoxia: consequences for living resources and ecosystems, Coastal and Estuarine studies 58. American Geophysical Union, Washington, DC, 454 pp

Ray RT, Haas LW, Sieracki ME (1989) Autotrophic picoplankton dynamics in a Chesapeake Bay sub-estuary. Mar Ecol Prog Ser 52:273–285

Redfield AC (1958) The biological control of chemical factors in the environment. Am Sci 46:205–222

Reed RH, Borowitzka LJ, Mackay MA, Chudek JA, Foster R, Warr SRC, Moore DJ, Stewart WDP (1986) Organic solute accumulation in osmotically stressed cyanobacteria. FEMS Microbiol Lett 39:51–56

Reynolds CS (1987) Cyanobacterial water blooms. Adv Bot Res 13:67–143

Reynolds CS (2006) The ecology of Phytoplankton. Cambridge University Press, Cambridge, UK, 535 pp

Reynolds CS, Walsby AE (1975) Water blooms. Biol Rev 50:437–481

Richardson K (1997) Harmful or exceptional phytoplankton blooms in the marine ecosystem. Adv Mar Biol 31:302–385

Robarts RD, Zohary T (1987) Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. NZ J Mar Freshw Res 21:391–399

Rocap G, Distel DL, Waterbury JB, Chisholm SW (2002) Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. Appl Environ Microbiol 68:1180–1191

Rueter J, Hutchins DA, Smith RW, Unsworth NL (1992) Iron nutrition of *Trichodesmium*. In: Carpenter EJ, Capone DG, Rueter JG (eds) Marine pelagic cyanobacteria: *Trichodesmium* and other Diazotrophs. Kluwer Academic Publishers, Dordrecht, pp 289–306, 357 pp

Ryther JH, Dunstan WM (1971) Nitrogen, phosphorus and eutrophication in the coastal marine environment. Science 171:1008–1112

Sañudo-Wilhelmy SA, Kustka AB, Gobler CJ, Hutchins DA, Yang M, Lwiza K, Burns J, Capone DG, Raven JA, Carpenter EJ (2001) Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. Nature 411:66–69

Schindler DW (1975) Whole-lake eutrophication experiments with phosphorus, nitrogen and carbon. Verh Int Ver Theor Angew Limnol 19:3221–3231

Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson M, Beaty KG, Lyng M, Kasian SEM (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc Natl Acad Sci USA 105:11254–11258

Schopf WJ (1993) Microfossils of the Early Archean Apex Chert: new evidence of the antiquity of life. Science 260:640

Seitzinger SP, Giblin AE (1996) Estimating denitrification in North Atlantic continental shelf sediments. Biogeochemistry 35:235–259

Sellner KG (1997) Physiology, ecology, and toxic properties of marine cyanobacteria blooms. Limnol Oceanogr 42:1089–1104

Seward AC (ed) (1909) Darwin and modern science. Essays in commemoration of the centenary of the birth of Charles Darwin and of the fiftieth anniversary of the publication of The origin of species. Cambridge: Cambridge University Press

Shapiro J (1990) Current beliefs regarding dominance of blue-greens: the case for the importance of CO_2 and pH. Verh Int Ver Theor Angew Limnol 24:38–54

Sharif DI, Gallon J, Smith CJ, Dudley E (2008) Quorum sensing in Cyanobacteria: *N*-octanoyl-homoserine lactone release and response, by the epilithic colonial cyanobacterium *Gloeothece* PCC6909. ISME J 2:1171–1182

Smetacek V, Bathmann U, Nöthig E-M, Scharek R (1991) Coastal eutrophication: causes and consequences. In: Mantoura RCF, Martin J-M, Wollast R (eds) Ocean margin processes in global change. Wiley, Chichester, pp 251–279

Smith VH (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221:669–671

Smith VH (1990) Nitrogen, phosphorus, and nitrogen fixation in lacustrine and estuarine ecosystems. Limnol Oceanogr 35:1852–1859

Staal M, Meysman FJR, Stal LJ (2003) Temperature excludes N_2-fixing heterocystous cyanobacteria in the tropical oceans. Nature 425:504–507

Stanier RY, Cohen-Bazire G (1977) Phototrophic prokaryotes: the cyanobacteria. Annu Rev Microbiol 31:225–274

Stein JR (1973) Phycological methods. Cambridge University Press, Cambridge, 448 pp

Stewart I, Falconer IR (2008) Cyanobacteria and cyanobacterial toxins. In: Walsh PJ, Smith SL, Fleming LE, Solo-Gabriele HM, Gerwick WH (eds) Oceans and human health: risks and remedies from seas. Academic, New York, pp 271–296

Stow CA, Borsuk ME, Stanley DW (2001) Long-term changes in watershed nutrient inputs and riverine exports in the Neuse River, North Carolina. Water Res 35:489–499

Stüken A, Rücker J, Endrulat T, Preussel K, Hemm M, Nixdorf B, Karsten U, Wiedner C (2006) Distribution of three alien cyanobacterial species (Nostocales) in northeast Germany: *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides*. Phycologia 45:696–703

Subramaniam A, Brown CW, Hood R, Carpenter EJ, Capone DG (2001) Detecting *Trichodesmium* blooms in SeaWiFS imagery. Deep Sea Res 49:107–121

Suikkanen S, Laamanen M, Huttunen M (2007) Long-term changes in summer phytoplankton communities of the open northern Baltic Sea. Estuar Coast Shelf Sci 71:580–592

Sylvan JB, Dortch Q, Nelson DM, Maier Brown AF, Morrison W, Ammerman JW (2006) Phosphorus limits phytoplankton growth on the Louisiana shelf during the period of hypoxia formation. Environ Sci Technol 40:7548–7553

Taylor FJR (1982) Symbioses in marine microplankton. Ann Inst Oceanogr (Paris) 58:61–90

Ter Steeg PF, Hanson PJ, Paerl HW (1986) Growth-limiting quantities and accumulation of molybdenum in *Anabaena oscillarioides* (cyanobacteria). Hydrobiologia 140:143–147

Thomas WH, Gibson CH (1990) Quantified small-scale turbulence inhibits a red tide dinoflagellate, *Gonyaulax-Polyedra* Stein. Deep Sea Res A 37:1583–1593

Tilman D, Kiesling RL (1984) Freshwater algal ecology: taxonomic tradeoffs in the temperature dependence of nutrient competitive abilities. In: Klug MJ, Reddy CA (eds) Current perspectives in microbial ecology. American Society for Microbiology, Washington, DC, pp 314–320

Tonk L, Bosch K, Visser PM, Huisman J (2007) Salt tolerance of the harmful cyanobacterium *Microcystis aeruginosa*. Aquat Microb Ecol 46:117–123

Vahtera E, Laanemets J, Pavelson J, Huttunen M, Kononen K (2005) Effect of upwelling on the pelagic environment and bloom-forming cyanobacteria in the western Gulf of Finland, Baltic Sea. J Mar Syst 58:67–82

Villareal TA (1992) Marine nitrogen-fixing diatom-cyanobacteria symbioses. In: Carpenter EJ, Capone DG, Rueter JG (eds) Marine pelagic cyanobacteria, *Trichodesmium* and other Diazotrophs. Kluwer Academic Publishers, Dordrecht, pp 163–175, 357 pp

Villareal TA (1994) Widespread occurrence of the *Hemiaulus*-cyanobacteria symbiosis in the southwest North Atlantic Ocean. Bull Mar Sci 54:1–7

Villareal TA, Carpenter EJ (1990) Diel buoyancy regulation in the marine diazotrophic cyanobacterium *Trichodesmium thiebautii*. Limnol Oceanogr 35:1832–1837

Vitousek PM, Mooney HA, Lubchenko J, Mellilo JM (1997) Human domination of Earth's ecosystems. Science 277:494–499

Vollenweider RA, Kerekes JJ (1982) Eutrophication of waters: monitoring, assessment and control. OECD, Paris

Vollenweider RA, Marchetti R, Viviani R (eds) (1992) Marine coastal eutrophication. Elsevier Science, New York

Walsby AE (1974) The extracellular products of *Anabaena cylindrica* Lemm. I. Isolation of a macromolecular pigment-peptide complex. Br Phycol J 9:371–381

Walsby AE (1992) The gas vesicles and buoyancy of *Trichodesmium*. In: Carpenter EJ, Capone DG, Rueter JG (eds) Marine pelagic cyanobacteria: *Trichodesmium* and other Diazotrophs. Kluwer Academic Publishers, Dordrecht, pp 141–161, 357 pp

Walsh JJ, Steidinger KA (2001) Saharan dust and Florida red tides: the cyanophyte connection. J Geophys Res 106:11597–11612

Waterbury JB, Rippka R (1989) Cyanobacteria. Subsection I. Order Chroococcales Wettstein 1924, emend. Rippka et al., 1979. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore, pp 1728–1729

Wiedner C, Rücker J, Brüggemann R, Nixdorf B (2007) Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. Oecologia 152:473–484

Wolk CP (1982) Heterocysts. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 359–386, 688 pp

Yunes JS, Salomon PS, Matthiensen A, Beattie KA, Raggett SL, Codd GA (2004) Toxic blooms of cyanobacteria in the Patos Lagoon Estuary, southern Brazil. J Aquat Ecosyst Stress Recovery 5:223–229

Zehr JP, Paerl HW (2008) Molecular ecological aspects of nitrogen fixation in the marine environment. In: Kirchman D (ed) Microbial ecology of the oceans. Academic, New York, pp 481–525

Zehr JP, Waterbury JB, Turner PJ, Montoya J, Omoregie E, Steward GF, Hansen A, Karl DM (2001) Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. Nature 412:635–638

Zehr JP, Methé B, Foster R (2005) New nitrogen-fixing microorganisms from the oceans: biological aspects and global implications. In: Wang YP, Lin M, Tian ZX, Elmerich C, Newton WE (eds) Biological nitrogen fixation, sustainable agriculture and the environment. Springer, Dordrecht, pp 361–365

Zehr JP, Montoya JP, Jenkins BD, Hewson I, Mondragon E, Church MJ, Hansen A, Karl DM (2007) Experiments linking nitrogenase gene expression to nitrogen fixation in the North Pacific subtropical gyre. Limnol Oceanogr 52:169–183

Physiology, Blooms and Prediction of Planktonic Cyanobacteria

6

Roderick L. Oliver, David P. Hamilton, Justin D. Brookes and George G. Ganf

Contents

R.L. Oliver (✉)
CSIRO Land and Water, CSIRO, PMB2, Glen Osmond, SA 5064, Australia
e-mail: Rod.Oliver@csiro.au

D.P. Hamilton
Environmental Research Institute, The University of Waikato, Hamilton, New Zealand
e-mail: d.hamilton@waikato.ac.nz

J.D. Brookes • G.G. Ganf
School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia
e-mail: Justin.brookes@adelaide.edu.au; George.ganf@adelaide.edu.au

Summary

This chapter addresses some of the challenges associated with trying to model population fluctuations, bloom formation and collapse of planktonic cyanobacteria. It is argued that improved modelling and prediction rely on a better understanding of the physiological responses of cyanobacteria to the physical and chemical characteristics of their environment. In addition there is a need to better understand the complex trophic interactions that influence population dynamics. The high variability of cyanobacterial populations represents a major challenge for models attempting to make predictions at the whole lake scale. Many of the physiological attributes described within specific models do not capture the dynamics of cyanobacteria, because of the extensive parameterisations required by the array of descriptive algorithms. The physiological attributes to be modelled include the ability to fix nitrogen, storages of both nitrogen and phosphorus, capture light across a range of wavelengths with

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specific accessory pigments, form colonies or filaments, and regulate buoyancy through the balance between gas vacuoles and cellular constituents. Recruitment of populations from sediments may also be important in bloom formation, but is not considered in this chapter. Although there is a commonality in models of cyanobacteria and micro-algae with their descriptions of photosynthesis, nutrient uptake, movement and grazing, there is a need to differentiate the cyanobacteria based on their key attributes, if their occurrence and succession are to be predicted separately from the micro-algae. The challenge is to develop models that incorporate complex physiological processes, responsive to changes at a range of ecosystem scales, but without excessive calibration of the key underlying algorithms. One suggestion is to turn from the single limiting-factor modelling approach that creates a plethora of disjointed algorithms and develop bio-mechanistic representations of integrated cellular function that incorporate dynamic responses to multiple effectors.

6.1 Introduction

6.1.1 Cyanobacterial Blooms

Cyanobacteria are ancient organisms (Schopf and Packer 1987; Chap. 2) whose blooms have been recorded through historical times (Francis 1878; Codd et al. 1994). Cyanobacterial blooms have detrimental effects on the quality of water supplies, partially due to the large biomass that can develop, because of the production of compounds that affect water flavour (Izaguirre et al. 1982) and also because of the risk of the toxins that are produced by some of the bloom forming species (Falconer et al. 1983; Carmichael 1992; Codd 1995; Azevedo et al. 2002).

The physiological adaptations that cyanobacteria have evolved to scavenge for limiting resources have proved highly successful in allowing them to occupy a niche in the pelagic zone of lakes (Vincent 2009). Increased occurrences of cyanobacterial blooms are frequently related to human influences in modifying the physical and biogeochemical conditions of aquatic systems (Reynolds 1987; Oliver and Ganf 2000). A shift in the phytoplankton community composition to more frequent dominance by cyanobacteria also alters the structure and trophic functionality of aquatic ecosystems by changing the flow paths of energy and nutrients.

An ability to predict the occurrence and extent of cyanobacterial blooms relies on developing models that can describe the growth, losses and distribution of planktonic cyanobacteria in response to different or changing environmental conditions. Benthic stages, including overwintering of vegetative cells (Chap. 7) or production of akinetes and their subsequent germination (Faithful and Burns 2006), may also be an important part of the life cycle of cyanobacteria, but knowledge of the environmental attributes that influence these stages is rudimentary and application of mechanistic models of these processes would be premature and is therefore not included in this chapter. Suitable models could provide the early warnings required by water resource managers to activate alleviation strategies including enhanced treatment processes and alternative water supplies (Ferguson 1997). There is also the expectation that improved model predictions might help identify new means of reducing bloom occurrences. Understanding how altered environmental conditions enhance the development of cyanobacterial blooms is also important for efforts aimed at restoring the community structure and trophic functionality of modified aquatic ecosystems.

The bloom-forming cyanobacteria are part of the diverse group of phototrophs that comprise the phytoplankton and are influenced by the same array of environmental conditions as their more taxonomically diverse counterparts, the eukaryotic micro-algae. Like the micro-algae, they are oxygenic phototrophs and capture most of their energy from sunlight, using this energy source to drive nutrient uptake and cellular metabolism that results in cell maintenance and growth. They are also impacted by losses similarly to the eukaryotic micro-algae, through depletion of nutrients and energy, grazing, sedimentation and microbial attack. It is not surprising that the approaches used in modelling the growth, physiology and ecology of cyanobacteria parallel those developed for planktonic micro-algae (e.g. Robson and Hamilton 2004). However, the prokaryotic cyanobacteria differ significantly from their eukaryotic counterparts in many aspects and these differences need to be incorporated into models that not only simulate cyanobacteria populations separately from eukaryotic populations but may also provide differentiation of individual populations of cyanobacteria. In this chapter cyanobacterial physiology is reviewed to elucidate the important processes, from the level of genes to populations, which need to be modelled to advance understanding of the complex interactions between hydrodynamics, biogeochemistry and cyanobacterial physiology.

6.1.2 Modelling

A complete understanding of the causal factors leading to cyanobacterial blooms is lacking. Hence a number of different modelling techniques have been adopted to predict the timing, spatial distribution and magnitude of cyanobacterial blooms, and to derive knowledge about their dynamics. None of these models is perfect and, so long as there is argument about how blooms come about, there will not be a definitive model. There has been considerable debate about the approaches used in modelling phytoplankton dynamics (e.g. Flynn 2003a, 2005). The current debate has been stimulated by acknowledgement that many of the current ecosystem models are based around outdated paradigms of phytoplankton physiology. Progress has not corresponded with what might

be expected from the exponential improvement in computing power, and new information on cellular functioning that has come from molecular approaches (Bhaya et al. 2000) and significantly enhanced measuring equipment (Flynn 2005). Such new developments, often described by explicit models of specific processes, have not been captured in comprehensive models of phytoplankton dynamics at larger (e.g. lake) scales or within the trophic structure of the aquatic system.

Much of the debate has also centred on empirical versus mechanistic models, a discussion that has been ongoing for decades. Modelling approaches have lagged behind the progress in allied fields (Zhao et al. 2008). Part of the debate revolves around the need to balance simplicity and realism, often resulting in empirical models (Flynn 2005), but in some cases the call for more detailed modelling has led to highly complex formulations for which the parameters may be difficult to assess and not easily extended to the system scale. Functional representations of key processes may provide the tools with which to link small-scale and large-scale representations (Zhao et al. 2008). The need for process focussed models becomes particularly evident when considering the varying responses to environmental conditions of different taxonomic and functional groups of phytoplankton. The aim of the models is to capture the competitive potential of different phytoplankton species or groups and therefore the seasonal sequences and shifts in community composition, including responses to major environmental alterations such as eutrophication and climate change (Brookes and Carey 2011; Kosten et al. 2012). Good process based models of phytoplankton will differentiate the characteristics of cyanobacteria from those of other phytoplankton, particularly characteristics that may contribute to blooms of cyanobacteria (Robson and Hamilton 2004). Such characteristics include buoyancy regulation, nutrient storage capacity, the capability to absorb light of different wavelengths, and the cellular balances between energy capture, nutrient uptake, nutrient assimilation, cellular composition and cellular metabolism. Different cyanobacterial taxa also vary in their relative capacity and efficiency in relation to these processes. For instance, it is known qualitatively how variations in nitrogen and phosphorus species and supply, stratification and light availability allow different cyanobacteria taxa to dominate (Carey et al. 2012), however the current suite of mechanistic models is severely challenged when presented with predicting the dominance of particular cyanobacterial taxa, due to limitations in the level of model process description and differentiation amongst the different taxa.

There are two main categories of models that have been used for simulating cyanobacteria: deterministic mathematical models and artificial neural network models (Guven and Howard 2006). The latter group includes a variety of modelling techniques such as genetic algorithms, Bayesian belief networks (Hamilton et al. 2007), fuzzy logic (Laanemets et al. 2006), and machine learning techniques that are designed to progressively adapt knowledge and statistical tools to an observed data set, and therefore to gradually reduce the error in predictions. In the case of Bayesian belief networks there is a judgment call developed through shared experience, in order to allocate a probability for the formation of blooms. Arhonditsis et al. (2007) provided an example of a coupled deterministic-Bayesian model in using a Bayesian calibration process to derive parameter values for a deterministic model. In some cases artificial neural networks have also been combined with deterministic models of lake hydrodynamics to capture the way in which cyanobacteria are affected by water mixing and transport processes (Ibelings et al. 2003). This chapter does not present details of artificial neural network models, but instead focuses on mathematical models with equations based around empirical or process representations of present knowledge of the ecology and physiology of cyanobacteria.

As part of our description of mathematical models of cyanobacteria we deal only briefly with hydrodynamic models. The reader is best advised to consult literature on physical limnology (Imberger and Patterson 1990; Imboden 2004), modelling of hydrodynamics (Hodges et al. 2000) and applications of coupled hydrodynamic-ecological models for cyanobacteria biomass prediction (Ibelings et al. 2003; Robson and Hamilton 2004; Hense and Burchard 2009) to understand the way these models represent hydrodynamics and are coupled with ecological models.

A major challenge for any model to simulate successfully the formation and magnitude of a bloom is to have a spatial scale suitable to encompass the variations in cyanobacterial concentrations within the waterbody. The difficulties in taking accurate measurements of cyanobacteria concentrations make this a particular challenge. Surface blooms are often most pronounced in calm conditions or on leeward shores under low wind speeds. Because wide variations can occur on space scales of a few millimetres (Fig. 6.1) to hundreds of metres (Fig. 6.2) and across whole lakes (Fig. 6.3), different sampling techniques can bias observations (Ahn et al. 2008). These depend, for instance, on the position and volume of sample collected or, in the case of *in vivo* fluorometry, the particular water mass sampled by the fluorometer. Furthermore, the usual quantitative measure of biomass is to collect a volume of water, either at a specific point in a waterbody or integrated with respect to depth, and then to make cell counts and possibly dimensional analyses of different species via light microscopy, to provide information on cell concentrations and biovolumes. Such a tedious and time-consuming process offers no opportunity to match the gridded nature of outputs from 3-D models and provides only limited capacity to validate 3-D coupled hydrodynamic-ecological model used to simulate cyanobacteria biomass. However, there are techniques that can markedly increase

sampling ability in a waterbody. For instance, flow cytometry can provide high throughput of water samples. Some fluorometers exploit the differences in spectral fluorescence associated with specific cyanobacterial pigments (phycocyanin or phycoerythrin), providing greater flexibility in interpreting the results than solvent-extracted chlorophyll *a* or chlorophyll fluorescence alone. Confidence in the results requires comprehensive calibration of the sensors.

Descriptions of mechanistically based phytoplankton models have been produced and a call has been made to create more unified modelling frameworks (Baumert and Petzoldt 2008; Mooji et al. 2010). This would help integrate efforts to develop comprehensive models. This approach has the added advantage of identifying the experimental and field studies needed to provide measurements to support the modelling, and perhaps lead to an international cooperative programme to provide the information (Mooji et al. 2010). It could also help to develop confidence in model predictions outside the dataset and ranges used for the initial model application and calibration.

6.2 Light Capture and Photosynthesis

Cyanobacteria carry out oxygenic photosynthesis in a manner similar to that found in the chloroplasts (Tandeau de Marsac and Houmard 1993). Although the chlorophyll *a* containing reaction centres of Photosystem I (PSI) and Photosystem II (PSII) are similar in cyanobacteria and microalgae, the major antennae, or light harvesting complexes

Fig. 6.1 Variability of cyanobacteria blooms in space: the small-scale: (all in New Zealand except for **c**.) (**a**) Lake Ohinewai, Waikato (A. Daniel); (**b**) Lake Rotorua (C. Zhang); (**c**) Lake Mendota, Wisconsin, USA (C. Spillman); (**d**) Lake Rotoehu, Rotorua (M. Landman); (**e**) Lake Ngaroto, Waikato (W. Powrie); (**f**) Lake Rotoroa, Waikato (W. Paul); (**g**) Lake Rotoiti, Rotorua (N. Miller); (**h**) Lake Ngaroto, Waikato (All photos with permission)

Fig 6.1 (continued)

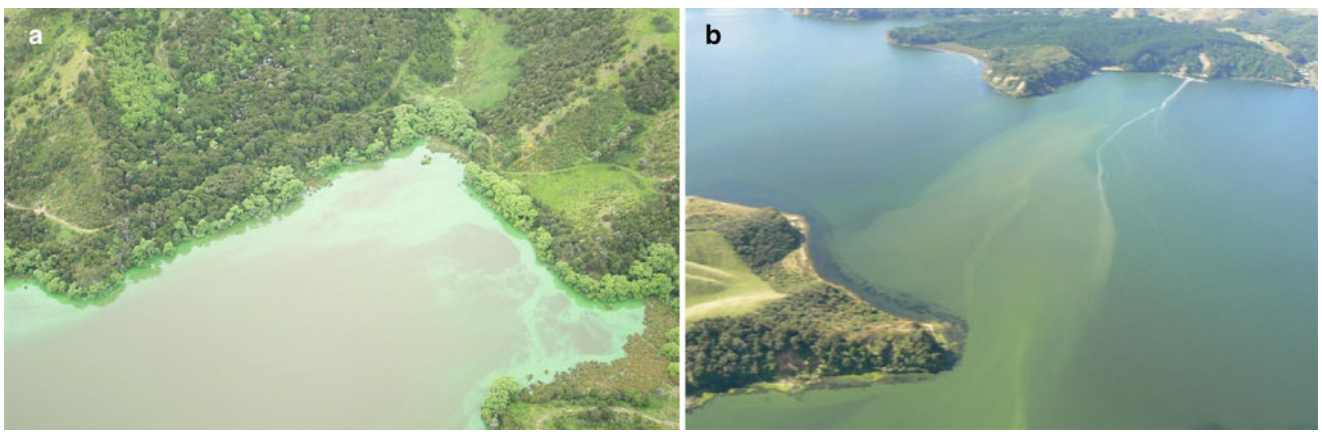


Fig. 6.2 Variability of New Zealand cyanobacteria blooms in space: aerial photos: (**a**) Lake Rotorua, Nelson; (**b**) Lake Rotoehu, Rotorua (Photos with permission: **a** = S.Wood; **b** = P. Scholes)

(LHC), that capture the incident photosynthetically active radiation (PAR) are quite different (Ormerod 1992; Grossman et al. 1995). In the micro-algae the antenna is integral to the thylakoid membrane and comprised largely of accessory chlorophylls. In cyanobacteria, chlorophyll *a*-protein complexes, photosynthetic reaction centres, carotenoids and the electron transport system are all contained within the thylakoids, but the major light-harvesting pigments, the

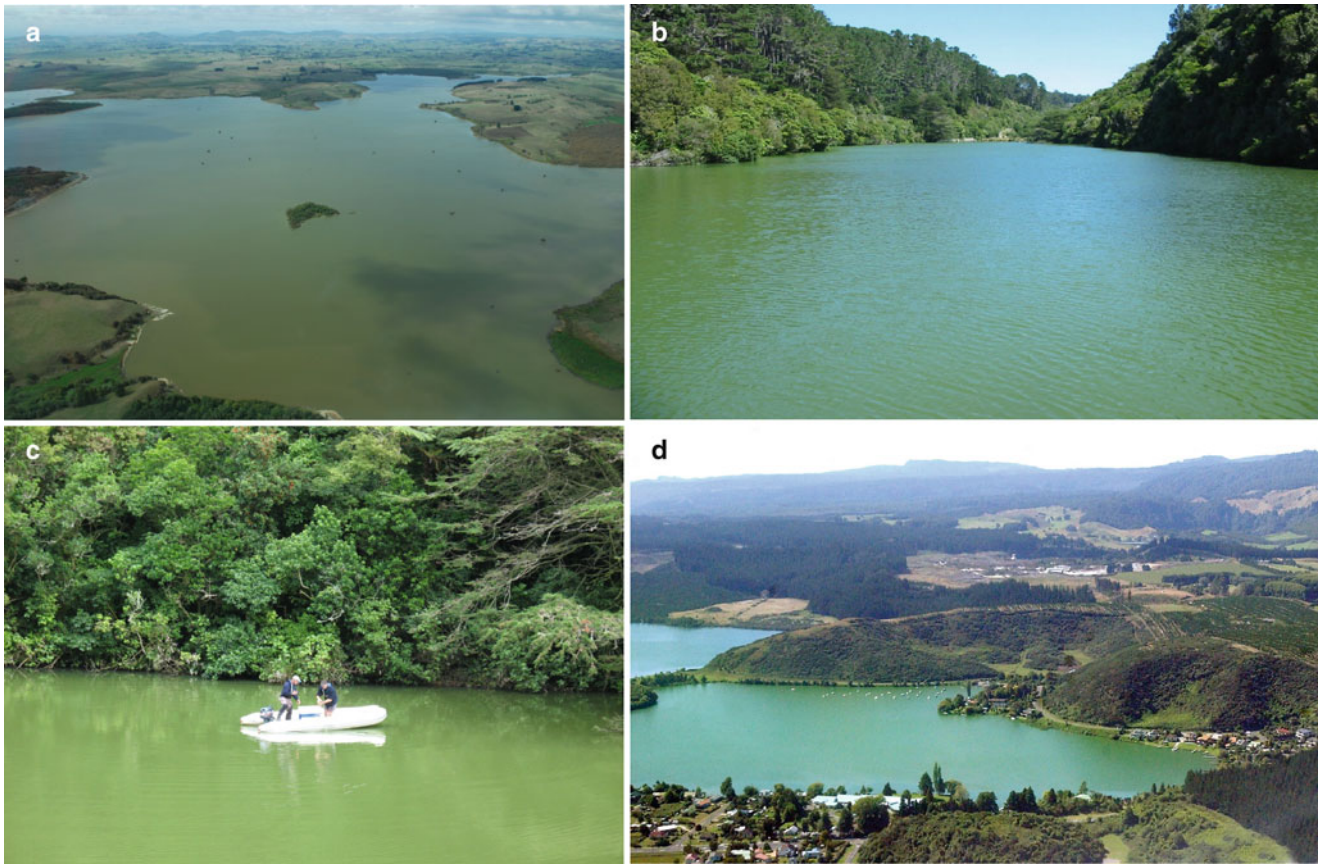


Fig. 6.3 Variability of New Zealand cyanobacteria blooms in space: the whole-lake scale: (**a**) Lake Whangape, Waikato; (**b** and **c**) Karori Reservoir, Wellington; (**d**) Okawa Bay of Lake Rotoiti, Rotorua (Photo of (**a**) by A. Zhang, with permission)

phycobiliproteins, occur within distinct light harvesting complexes called phycobilisomes (PBS) (Adams and Duggan 1999). The PBS form rows of hemidiscoidal structures attached to the surface of the thylakoids (Bryant 1991). Each PBS is made up of pigmented phycobiliproteins that form a series of rods connected to the PBS core. The phycobilin chromophores associated with the rods are phycocyanin (A_{max} 620 nm) and the red pigmented phycoerythrin (A_{max} 560 nm), and these, in conjunction with the PBS core of allophycocyanin (A_{max} 650 nm), determine the light absorption spectra. Whereas all PBS contain allophycocyanin and phycocyanin, only some contain phycoerythrin. It is in species with such PBS that alterations in pigment composition can have their greatest effect, changing blue-green cells to red as they adapt to different light spectra, the process of chromatic adaptation (Tandeau de Marsac and Houmard 1993; Stomp et al. 2004).

The unidirectional flow of energy in the PBS is from the rods, through the core and then via linker polypeptides and terminal electron acceptors to the photosystem reaction centres (Bailey and Grossman 2008). Electrons are generated from the light dependent oxidation of water by the oxygen evolving complex of PSII, and transported through the electron transport chain to PSI. Plastoquionone (PQ) is first reduced and then electrons are passed via cytochrome b6 f complex and plastocyanin to PSI, resulting in the reduction of ferredoxin and $NADP^+$ (Fig. 6.4). This generates energy and reductant for cellular metabolism and carbon fixation (Singh et al. 2009; Alric et al. 2010). Both of the connected photosystems harvest light energy, but of different wavelengths, as the phycobiliproteins in the PBS are largely associated with PSII, while chlorophylls, that have maximum absorbance wavelengths of 435 and 680 nm, are largely associated with PSI (Singh et al. 2009).

6.2.1 Spectral Influence

The underwater light climate varies in quantity and quality with depth. The intensity decreases exponentially as a result of absorption and scattering by particles and coloured compounds, and the selective removal of wavelengths causes shifts in the spectral distribution (Kirk 1983; Oliver 1990; Oliver and Ganf 2000; Stomp et al. 2007). Water absorbs strongly in the red, so that in marine systems and very clear

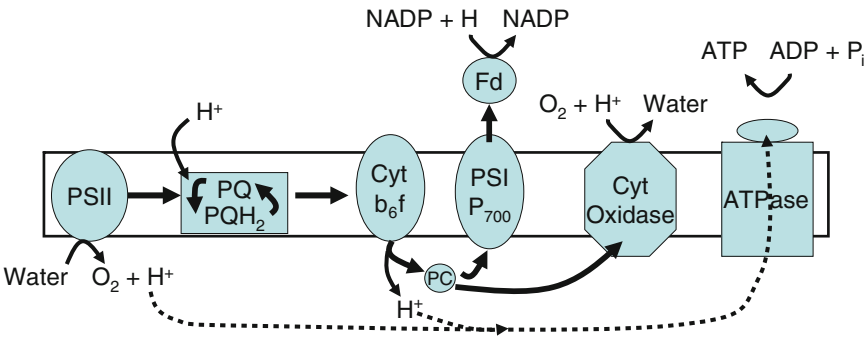


Fig. 6.4 Photosynthetic linear electron transfer pathways in the thylakoids. Photosystem II (PSII), Photosystem I (PSI), Primary electron donor of PSI (P_{700}), Cytochrome b_6f (Cyt b_6f), Plastocyanin (PC), Ferredoxin (Fd), Respiratory terminal oxidase (Cyt Oxidase), Proton pumping for ATP generation (ATPase)

inland waters where the majority of light attenuation is due to the water, the irradiance becomes dominated at depth by shorter wavelengths. In contrast, many inland and coastal waters contain sufficient dissolved organic compounds and suspended particles to absorb strongly in the blue, causing a shift towards longer wavelengths with depth (Kirk 1983; Oliver 1990; Oliver and Ganf 2000; Stomp et al. 2007). Phytoplankton also modifies the spectral distribution of light. For example, green algae use carotenoids and chlorophyll *a* and *b* to absorb light in the red and blue region, whereas they only weakly absorb green and yellow light (525–650 nm range) leaving an orange-green window of irradiance (Kirk 1983; Stomp et al. 2007). These wavelengths are suitable for absorption by the phycobiliproteins and as a result micro-algae may modify the spectral distribution of light at depth to the advantage of cyanobacteria.

The phycobiliproteins absorb PAR over a much wider range of wavelengths than the antennae of the micro-algae (Glazer et al. 1994), particularly in the region between the absorption bands of the accessory chlorophylls b and c, and the carotenoids. This difference between cyanobacteria and eukaryotic micro-algae is likely to be a distinct advantage where either the spectral quality of the underwater light is concentrated in these wavebands, or when there are substantial fluctuations in light quality over time. It might be expected from these comparisons of spectral absorption characteristics that the cyanobacteria would respond quite differently from micro-algae to changes in PAR. This differentiation has not been included in models to distinguish biomass of cyanobacteria from micro-algae in natural systems.

Huisman et al. (1999) measured the critical light intensity required to sustain continuous monocultures of two cyanobacteria (*Microcystis* and *Aphanizomenon*) and two eukaryotic micro-algae (*Chlorella* and *Scenedesmus*). In competition for light the species with the lowest critical light requirement should be the superior competitor. However, in mixed cultures the critical light intensities were different from those measured in monocultures and this altered the respective competitive abilities. This change was attributed to alterations in the spectral distribution of light in the mixed cultures where green algae shifted the light spectrum to green and yellow light that could be absorbed by the phycobilin pigments of the cyanobacteria (Huisman et al. 1999; Stomp et al. 2007). In the competition experiments *Chlorella* displaced all three other species, *Microcystis* displaced both *Aphanizomenon* and *Scenedesmus,* and *Aphanizomenon* only displaced *Scenedesmus.* These findings do not support suggestions that cyanobacteria are better adapted to low light conditions and hence better competitors for light than are green algae (Mur 1983; Richardson et al. 1983). However, in these experiments the light source was white fluorescent tubes and the spectral distribution was modified only by the phytoplankton. It is likely that the outcome of these competition experiments would be different if the water contained particulate and dissolved materials that substantially altered the light spectrum (Oliver 1990; Kirk and Oliver 1995; Ganf et al. 1989; Stomp et al. 2007).

Wyman and Fay (1986) grew eight strains of cyanobacteria under equivalent photon fluxes of red, green, blue and white light and found large differences in the cell concentrations of photosynthetic pigments and in growth rates. In red light there was a decline in chlorophyll and phycobiliprotein content, but all strains grew at a significantly faster growth rate than under an equivalent photon flux of white light. Under green light the pigment composition was similar to that under white light, but only the two phycoerythrin-rich strains (*Oscillatoria agardhii*=*Planktothrix agardhii* and *Gloeotrichia echinulata*) grew significantly faster, all other strains growing at 60–75% of their white light rate. In blue light the pigment composition was again similar to that under white light although a majority of the phycocyanin-rich strains showed a reduction in chlorophyll content. The strains rich in phycocyanin had growth rates <50% of their white light rate, while the phycoerythrin-rich strains, *O. agardhii* and *G. echinulata*, were able to maintain growth rates of 65% and 100% of their white light growth rates, respectively.

Comparison in continuous culture of phycoerythrin-rich species with closely related green species devoid of phycoerythrin has further demonstrated the influence on competition of pigmentation and spectral changes (Stomp et al. 2004; Oberhaus et al. 2007). Stomp et al. (2004) compared a red and green species of *Synechococcus* and showed that although

one would dominate under red light and the other in green light, under white light they could coexist. They then compared these two species with *Tolypothrix tenuis*, a marine filamentous cyanobacterium that can undergo complementary chromatic adaptation by adjusting the ratio of its phycocyanin to phycoerythrin in response to spectral changes. It was found that this species could coexist with either of the *Synecochoccus* species by producing complementary pigments to absorb the alternative colour to that used by the competitor. Under white light *Tolypothrix* coexisted with the green *Synecocchocus* by increasing its phycoerythrin content and turning red. In competition with the red *Synecochcoccus, Tolypothrix* was reduced to low numbers but could not be excluded from the culture because it turned green by increasing its phycocyanin content and utilised the unabsorbed light. Such adaptive pigmentation changes are beyond the process descriptions used in current mechanistic models of phytoplankton dynamics.

6.2.2 Photoacclimation, Photoadaptation and Photoinhibition

To maintain their light harvesting efficiency and to avoid increased risks of photodamage when exposed to high irradiances, phytoplankton have developed mechanisms for adjusting to alterations in the intensity, spectral distribution and periodicity of the PAR supply (Falkowski and La Roche 1991). Photoacclimation describes changes in the overall photosynthetic apparatus to cope with the "average" photon supply that results from prolonged exposure to relatively consistent light conditions. It involves the degradation and synthesis of components of the photosystem including light harvesting pigments, reaction centre components and dark cycle intermediates. Two major strategies are employed for adjusting to irradiance intensity. The first involves alterations in the size of the light harvesting antennae that serve the photosystems, and the second is a change in the total number of photosynthetic units (Wyman and Fay 1986; Falkowski and La Roche 1991). If photosynthesis becomes limited by the rate of delivery of light energy to the photosystems, as under low irradiance, then an increase in antenna size provides one means of increasing the photon supply. If the supply of photons from the antenna approaches the maximum turnover rate of the photosystem, then an increase in the number of photosynthetic units will increase the total supply of energy to the cell for photosynthesis and growth (Falkowski and La Roche 1991). In general lower irradiances result in increased light harvesting and a decrease in electron transport and carbon fixation while higher irradiances have the reverse effect (Bailey and Grossman 2008).

Planktonic cyanobacteria can experience large and rapid changes in their light environment, for example as a result of vertical mixing. Short term light fluctuations are dealt with by short-term, reversible changes to the photosynthetic apparatus that reduce light capture and enhance the release of captured energy as heat rather than through photochemistry. These processes are generally referred to as photoadaptation. Although light is required to drive photosynthesis, excessive light can cause the electron flow to exceed the capacity of the electron transport chain and the downstream utilization of reducing equivalents. This has photo-damaging effects on the photosystem and also results in an imbalance in the redox state of the cell, affecting many other cellular processes including the utilization of nutrients and the activity of metabolic pathways (Aurora et al. 2007; Bailey and Grossman 2008). In cyanobacteria, as in other photoautotrophs, there is a need to regulate the excitation of PSII and PSI in response to light intensity and spectral quality. This is to balance the delivery of energy and maximise the quantum yield of the light reactions, and also to reduce the probability of photodamage (Singh et al. 2009). A number of mechanisms are involved in photoadaptation in cyanobacteria.

High levels of light can cause photoinhibition to PSII reaction centres. At the core of the PSII complex is a heterodimer of two homologous polypeptides D1 and D2. The D1 protein has a more rapid turnover than any other thylakoid or chloroplastic protein and is part of a cycle of damage and repair that is essential for maintenance of PSII function under photoinhibitory conditions (Bouchard et al. 2006). Under illumination the D1 protein degrades and re-synthesizes to limit accumulation of photodamaged PS II reaction centres (Bouchard et al. 2006). When the rate of repair matches the rate of photodamage then photoinhibition is not apparent. However if under increasing light intensity the rate of damage exceeds that rate of repair then photoinhibition occurs (Andersen 1997; Heraud and Beardall 2000; Han et al. 2000; Oliver et al. 2003; Bailey and Grossman 2008). The rate of repair can be negatively influenced by nutrient limitation and UVB light so that the onset of photoinhibition is not just a function of the light intensity (Bouchard et al. 2006).

The PBS of cyanobacteria are highly mobile and can associate or disassociate with PSII or PSI resulting in a process of state-transitions. The state transitions redirect energy between the two photosystems when changing light conditions disturb the energy balance. In cyanobacteria there is more chlorophyll (Chl) associated with PSI than PSII. Under light conditions that predominantly excite Chl (e.g. blue PSI light), cyanobacteria maintain the balance between photosystems by decreasing PBS energy transfer to PSI and increasing energy transfer to PSII. This is a state 2 to state 1 transition.

The state transition is controlled by the redox poise of the plastoquinone pool (PQ) which develops an increased oxidation level under conditions where photons are being directed to PSI. Conversely, when excessive energy is being directed to PSII (red PSII light) and the PQ is more reduced, a State 1 to State 2 transition occurs as the PBS increases energy transfer to PSI (Bailey and Grossman 2008; Singh et al. 2009).

In cyanobacteria, there appear to be at least two photoprotective mechanisms that reduce the transfer of excitation energy from the light-harvesting complexes to the photosynthetic reaction centres through the active dissipation of absorbed energy. The first mechanism is through a blue light induced soluble orange carotenoid protein (OCP) that is widely distributed among cyanobacteria species (Kerfeld 2004). It mediates photoprotective energy dissipation through interaction with the phycobilisome core (Bailey and Grossman 2008; Latifi et al. 2009). The second energy dissipation mechanism is related to the high light-inducible proteins (HLIPs), also designated small CAB-like proteins (SCPs) that may play a critical role in photoprotection by associating with Photosystem II and dissipating excess absorbed energy (Latifi et al. 2009).

6.3 Photosynthesis and Cellular Metabolism

The growth of cyanobacteria depends on light absorption, temperature, and the uptake of a range of nutrients (Behrenfeld et al. 2008) and these cellular functions need to be regulated in a coordinated manner (Aurora et al. 2007). Mechanisms to maximise growth must optimise the allocation of energy (ATP) and reductant (NADPH) between the various cellular processes in order to coordinate resource supply and demand. Assimilation of atmospheric CO_2 is through the Calvin Cycle and requires three ATP per two NADPH molecules, i.e., a ratio of 9:6 to form its product glyceraldehyde-3-phosphate (GAP) (Behrenfeld et al. 2008; Alric et al. 2010). Strictly linear electron transport from PSII to PSI produces these molecules in the ratio of 9:7, which although matching closely the Calvin Cycle requirements means that there is no ATP or NADPH left for other cellular activities. Conversely, the demand for ATP by other metabolic pathways unbalances the ATP to NADPH ratio required for CO_2 fixation by the Calvin Cycle. Photosynthesis is the source of ATP and reductant, and cyanobacteria have evolved multiple methods for enhancing ATP generation to balance overall supply and demand, generally through the conversion of reductant.

At night the ATP to reductant ratio is balanced by respiratory electron transport which uses NADPH to form ATP. But in cyanobacteria this balance is more difficult to maintain during the daylight hours due to a unique characteristic of these organisms. In cyanobacteria, components of the electron transport chain that reside in the thylakoid membrane are shared by both respiratory and photosynthetic electron transport. Consequently, during daylight hours, the electron transport chain is less available for respiration due to photo-reduction of some of its components and this can be further exacerbated by a lack of $NADP^+$ substrate for reduction. Under these conditions PSII activity is curtailed as the oxidation rates of the electron chain intermediaries are reduced due to the respiratory electron transport, a situation that can cause PSII energy absorption to exceed photochemistry, leading to photoinhibition. In order to overcome the imbalances in ATP and NADPH that occur under these conditions cyanobacteria use several mechanisms to regulate the balance between linear and cyclic electron flows (Alric et al. 2010).

Linear electron flow produces reducing power and ATP, while cyclic electron flow around PSI only produces ATP (Fig. 6.5). Low cellular requirements for ATP to NADPH favour coordinated linear electron flow through PSII and PSI. In this case, any requirement for extra ATP is produced through thylakoid water to water cycles like the Mehler reaction and the respiratory terminal oxidase that generate ATP by pumping protons across the thylakoid membrane. In comparison, high ATP to NADPH demands are thought to decouple PSII and PSI to create two distinct pathways (Behrenfeld et al. 2008). One pathway involves cyclic electron flow around PSI in which ferredoxin, reduced by PSI,

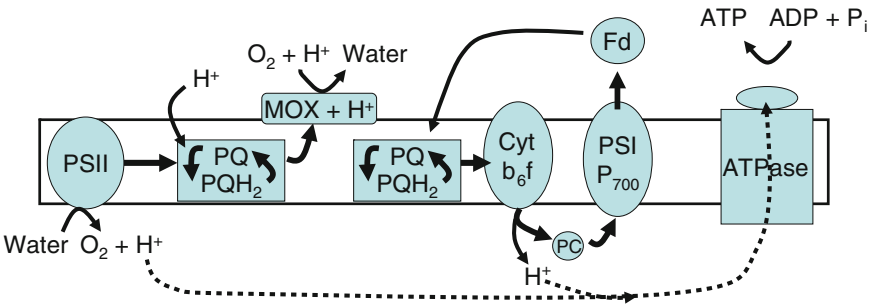


Fig. 6.5 Alternative photosynthetic electron transfer pathways that alter the balance of ATP and NADPH production (After Behrenfeld et al. 2008). Symbols additional to Fig. 6.1: Midstream terminal oxidases (MOX)

transfers electrons to the PQ pool which then transfers them to cyctochrome b6 f, plastocyanin and back to PSI to support ATP production. Secondly it has been suggested that cyclic electron flow around PSI is supported by 'midstream' terminal oxidases (MOXs) that augment ATP synthesis using electron flow from PSII, and this has the extra advantage of helping to reduce PSII photoinhibitory stress (Behrenfeld et al. 2008).

The requirement for a high or low ATP to NADPH ratio is determined by the metabolic activity of a cell so that balancing the relative supplies of energy and reductant involves not only adjustments in the photosystem, but also changes in cellular metabolism. Molecular and biochemical studies have provided insight to the homeostatic interactions between light capture, cellular metabolism and cell growth. Measurement of transcript abundance by DNA microarray shows that approximately 33% of genes in *Synechocystis* are regulated in response to changes in light quality (Singh et al. 2009). Analysis of these genes during changes in light quality that induced state transitions led to the identification of cellular processes that enable *Synechocystis* to circumvent reduced production of energy and reductant (Singh et al. 2009). That most cellular processes responded immediately to the imbalance in the excitation of reaction centres suggests that state transitions and adjustments of photosystem structure are not sufficient by themselves to reverse the effects of excitation imbalance (Singh et al. 2009).

The supply and demand for energy and reductant are determined by the extent to which ATP and NADPH from the photosystem are used in different metabolic pathways (Fig. 6.6). The synthesis of amino acids utilises GAP produced by photosynthesis, but the pathway releases carbon with a net production of ATP and NADPH to support other metabolic activities. So a significant proportion of energy and reductant from the photosystem can be channelled through this pathway when it is operating. In contrast, if GAP is used for carbohydrate synthesis and storage which does not release ATP and reductant, then only a fraction of the energy and reductant from the photosystem can be invested in this process as the rest is required to directly support other cellular metabolic functions (Behrenfeld et al. 2008).

The activity of these various metabolic pathways will also be influenced by nutrient availability. One example is nitrogen assimilation, where energetically NH_4^+ is preferential to NO_3^-, as the former requires far less energy for assimilation into glutamate (Coruzzi and Last 2000). The form of nitrogen used will influence the ATP:reductant demand ratio and alter the activity of different nitrogen assimilatory pathways (Behrenfeld et al. 2008).

Singh et al. (2009) found in *Synechocystis* that photodamage from high light decreased the output of products from the light reactions with the reduction in energy reducing CO_2 fixation. This in turn caused a reduction in N transport and assimilation. The reduced assimilation of C and N had consequences for various pathways, including those involved in transcription, translation, DNA replication, fatty acid metabolism, and biosynthesis of amino acid and nucleotides. They also found significant changes in response to light quality. Under PSII light *Synechocystis* was limited for NADPH and preferentially utilised ammonia over nitrate under these conditions. This was supported by experiments that showed the growth of *Synechocystis* increased significantly in the presence of ammonium under PSII light compared with white light (Singh et al. 2009). These connections mean that alterations in photosynthesis ramify through the cell, causing changes in stoichiometric composition and metabolic activity in a connected and coordinated way.

6.3.1 Modelling of Photosynthesis

Modelling of photosynthesis in cyanobacteria has utilised the same general functions that are applied to phytoplankton (Baklouti et al. 2006). In most cases empirical relationships have described the dependency of photosynthesis (P) on light in terms of the maximum rate of photosynthesis (P_{max}) and a function of irradiance (E) and a light saturation parameter (E_k):

$$P = P_{max} f\left(\frac{E}{E_k}\right)$$

Numerous different equations of this basic form have been fitted to experimental data (Table 1 in Baklouti et al. 2006). Some equations include additional parameters to describe photoinhibition, but these are also empirical formulations that provide "best fits" to the data (Jassby and Platt 1976). Even when describing the same data these various equations provide different estimates of the characteristics of the P-E curve including the initial slope (α), and the light saturation parameter (E_k) (MacIntyre et al. 2002; Baklouti et al. 2006). These parameters reflect the underlying biophysical and physiological processes that regulate photosynthesis (MacIntyre et al. 2002), but the standard empirical P-E data sets cannot explicitly and quantitatively describe these processes. As the equations usually describe empirical data from incubations under fixed or average conditions they are limited in their ability to describe photo-physiological adjustments or to predict changes in the P-E relationship under changing environmental conditions (Walsby et al. 2001).

Progress in modelling these interactions has come from advances in biochemical and molecular understanding, and also improvements in the measurement of cellular photophys-

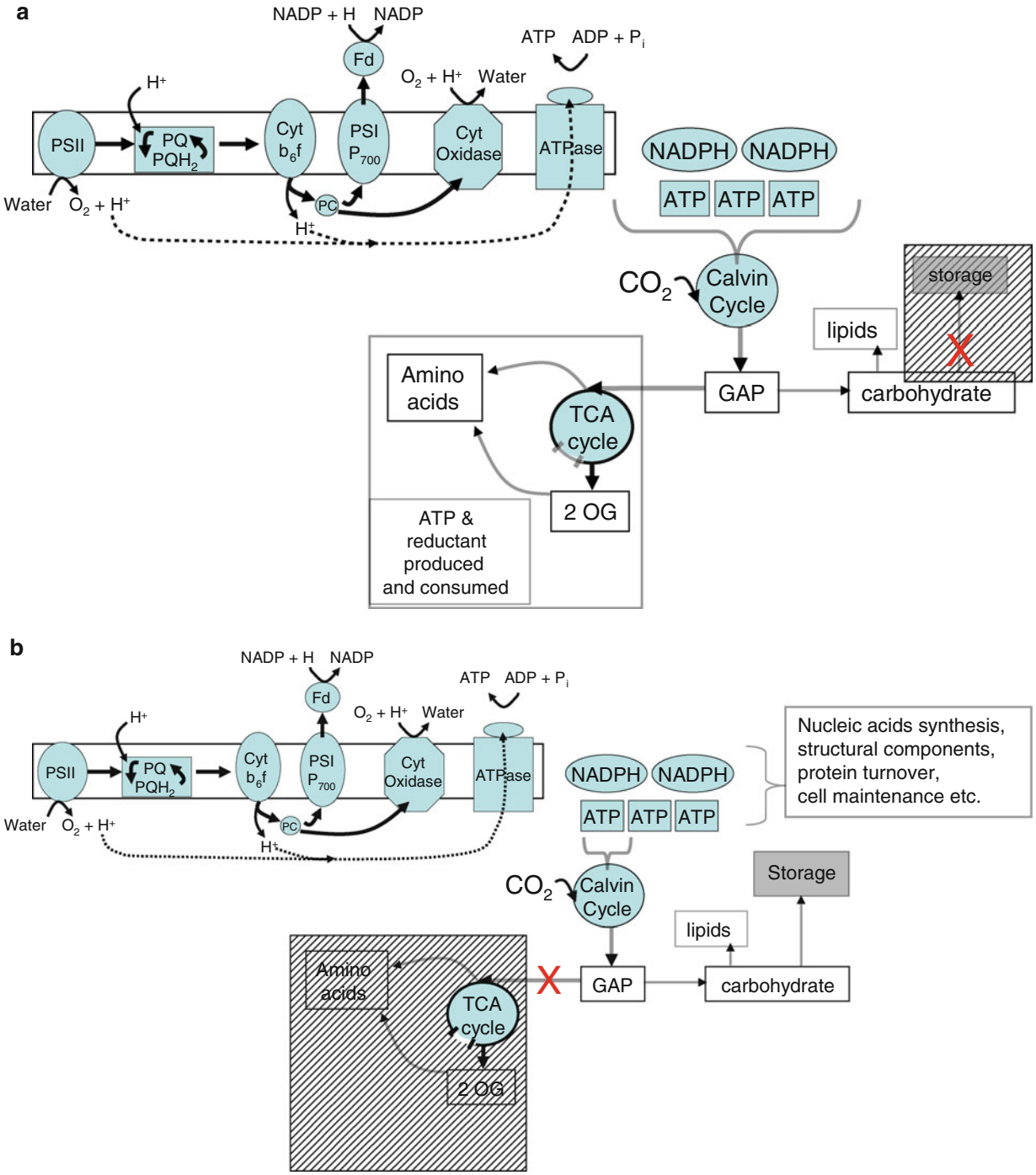


Fig. 6.6 Dominant metabolic pathways during (**a**) amino acid synthesis and (**b**) carbohydrate synthesis (After Behrenfeld et al. 2008). Symbols additional to Fig. 6.4: Glyceraldehyde-3-phosphate (GAP), Tricarboxylic acid cycle (TCA), 2-oxoglutarate (2-OG)

iology, especially through active fluorometry (Schreiber et al. 1995; Kolber and Falkowski 1993; Oliver and Whittington 1998). These techniques have led to process based models that include photophysiological characteristics such as the absorption cross-section of the photosynthetic units, electron transfer rates, and photo-acclimation rates (Falkowski and Kolber 1993; Han et al. 2000; Oliver et al. 2003). An equation for photosynthesis based on fluorescence measurements was derived by Falkowski and Kolber (1993):

$$P = E\sigma q_p \frac{\Phi'}{\Phi_m} \phi_e \eta_{PSII}$$

Here P is the rate of photosynthesis (mol O_2 mol chla^{-1} h^{-1}), E is the irradiance intensity (mol photons m^{-2} h^{-1}), σ is the effective absorption cross-section of the antennae which determines the photons actually reaching the PSII reaction centre, (m^2 mol quanta^{-1}), Φ'/Φ_m is the fraction of functional reaction centres, ϕ_e the mol of oxygen evolved per photon processed by the reaction centres, and η_{PSII} the number of functional PSII reaction centres per mole of chlorophyll-a (Falkowski and Kolber 1993). The effective absorption cross-section has a spectral dependence and this can be built into the equation though in general a spectrally integrated absorption coefficient is used. It will be important to improve the modelling of spectral effects to better estimate photosynthesis under changing light conditions and to improve the modelling of competition between species (Sathyendranath et al. 2007). Photoinhibition has also been included in some models of this type (Han et al. 2000; Oliver et al. 2003).

6.4 Nutrients

Nutrient limitation of cyanobacteria elicits both general and specific responses. The general responses are the result of the stresses imposed by arrested anabolism while specific responses are acclimation processes to particular nutrient limitations. Specific responses lead to modification of metabolic and physiological activities to compensate for the restriction (Schwarz and Forchhammer 2005). Nutrient limitation is frequently considered the cause of reduced growth of phytoplankton in natural environments and an important driver of competition that determines community composition. Considerable attention has been focused on nutrient limitation by phosphorus versus that by nitrogen, and the ratios of these nutrients have been used at whole lake scales to predict the relative abundance of cyanobacteria amongst lake phytoplankton (Sect. 6.8).

General responses of phytoplankton to nutrient limitation include: carbohydrate accumulation, a reduction in the cell-specific quantum yield of photosynthesis (Turpin 1991), a reduction in the cellular content of the limiting nutrient (Droop 1973; Riegman and Mur 1984) and an increase in the specific uptake rate of the limiting nutrient (Gotham and Rhee 1981; Riegman and Mur 1984; Kromkamp 1987). Nutrient limitation stimulates the storage of non-limiting nutrients as a result of their relative excess compared to the reduced requirements of the cell. Nutrient storage is a valuable attribute, enabling cells to utilise pools of nutrients that are spatially and temporally separated so that growth is maintained during periods of nutrient scarcity. Phytoplankton must match their energy input to their cellular metabolic capacity, and as nutrient limitation slows down the reoxidation of the final electron acceptors, electron transfer activity must be down-regulated and cellular metabolic pathways adjusted to minimise possible photodamage and to maximise energy efficiency.

6.4.1 Phosphorus

Under phosphorus limiting conditions cellular phosphorus concentrations decline as the phosphorus limited growth rate declines, while phosphorus uptake potential increases. As a consequence, a pulse of phosphorus delivered to P-limited cells results in substantial formation of polyphosphate reserves, the polyphosphate 'overplus' phenomenon, with cellular P levels able to exceed those under steady state maximum growth rates (Allen 1984; Riegman and Mur 1984). Most phytoplankton can store surplus phosphorus, usually in the form of polyphosphate (PP), and these reserves can be sufficient for several cell doublings. There do not seem to be any consistent phylogenetic differences between microalgae and cyanobacteria in the range of values for phosphate uptake, and the kinetics appear to be species specific (Healey 1982; Tilman et al. 1982; Kromkamp 1987; Reynolds 1993).

Despite its importance in eutrophication and its role in cellular energy dynamics surprisingly little detailed molecular or biochemical data are available on the cellular metabolism of P in cyanobacteria compared to that for N. This probably reflects the dominance of marine phytoplankton research in this area and the focus on N limitation in marine systems. This deficiency needs to be addressed.

6.4.2 Nitrogen

Cyanobacteria are able to utilise a range of N sources including ammonium, nitrate, nitrite, urea, and in some cases arginine or glutamine (Flores and Herrero 2005). Certain groups can fix atmospheric N_2, a trait that distinctly separates them from the autotrophic eukaryotes. The order of preference amongst the commonly available inorganic sources is $NH_4^+ > NO_3^- > N_2$ (Tandeau de Marsac and Houmard 1993). Energetically, NH_4^+ is preferential to NO_3^- as the former requires only one NAD(P)H or ferredoxin and one ATP for assimilation into glutamate, while the latter requires nine reductants and one ATP (Coruzzi and Last 2000). During the day, these substrates are provided directly from photosynthesis and thus the form of nitrogen used influences both the ATP:reductant demand ratio and the photosynthesis:carbon fixation ratio (Behrenfeld et al. 2008). In nutrient replete cells carbohydrate stores are small and assimilation of combined inorganic nitrogen is strongly dependent on recent CO_2 fixation (Guerrero and Lara 1987; Turpin 1991). Under these conditions reductions in photosynthesis, for example due to darkness or CO_2 deprivation, will reduce nitrogen assimilation.

In contrast, nitrogen limited cells accumulate carbohydrate reserves that can be utilised through glycolysis as a source of energy and carbon skeletons for nitrogen assimilation both in the dark and the light (Guerrero and Lara 1987; Turpin 1991; Garcia-Gonzalez et al. 1992; Tapia et al. 1996).

The uptake of nitrate/nitrite, urea and most amino acids usually involves permeases, while the uptake of ammonium involves secondary transporters. Within the cell, nitrate is converted to nitrite by nitrate reductase and then nitrite is converted to ammonium by nitrite reductase. Arginine is catabolized by a combination of the urea cycle and arginase pathway, while urea is degraded by a Ni^{2+}-dependent urease; both these pathways also produce ammonium (Flores and Herrero 2005).

Ammonium, derived from direct uptake or produced from conversion of other nitrogen sources, is incorporated into carbon skeletons through the glutamine synthetase–glutamate synthase cycle (GS-GOGAT). When NH_4^+, the preferred N source is available, its presence represses the genes encoding permeases and enzymes for the assimilation of alternative nitrogen sources and cyanobacteria and micro-algae do not assimilate these other forms of nitrogen. This process is known as 'nitrogen control' (Turpin 1991; Ochoa de Alda et al. 1996; Flores and Herrero 2005). Induction of ammonium inhibition of nitrate uptake requires that ammonium has first been metabolised by the initial glutamine synthetase step of the GS-GOGAT system (Herrero et al. 2001). The subsequent glutamate synthase step requires carbon skeletons and these are supplied as 2-oxo-glutarate, which is the final compound in the oxidative TCA cycle in cyanobacteria as they lack 2-oxo-glutarate dehydrogenase. Ammonium depletion limits GS-GOGAT activity and results in the accumulation of 2-oxo-glutarate, while large supplies of ammonium or restrictions to photosynthesis may lead to reduced concentrations of 2-oxo-glutarate (Schwarz and Forchhammer 2005). This metabolic arrangement where 2-oxo-glutarate consumption through GOGAT is directly linked to ammonium assimilation (Fig. 6.7), integrates the N and C assimilatory pathways of cyanobacteria and provides the basis of an important regulatory system where 2-oxo-glutarate is an indicator of the C to N ratio of the cells (Schwarz and Forchhammer 2005; Flores and Herrero 2005).

Two proteins responsive to 2-oxo-glutarate are the signal transducer protein PII and the nitrogen-control transcription factor NtcA. The protein PII binds both ATP and 2-oxo-glutarate in a synergistic manner to alter its reactivity (Forchhammer 2004). PII can exist in four different forms, non-phosphorylated and increasingly phosphorylated at one, two or three of its subunits. In high concentrations of 2-oxo-glutarate the PII is liganded to both ATP and 2-oxo-glutarate and in this form it can be phosphorylated while in reduced concentrations of 2-oxo-glutarate the PII is bound only to ATP and can be dephosphorylated. In ammonium-grown cells, where the high demand for carbon skeletons lowers the 2-oxo-glutarate concentration, PII is non-phosphorylated. The level of PII phosphorylation is increased in nitrate-grown cultures and higher again in nitrogen-starved cells, but this is not a simple monotonic progression as the level of phosphorylation increases when cells are incubated in the presence of CO_2 enriched air (Tandeau de Marsac et al. 2001; Schwarz and Forchhammer 2005; Kolodny et al. 2006). As a result, the degree of phosphorylation of PII is a function of the N and C supply of the cell such that phosphorylation is inversely correlated with nitrogen availability, but directly correlated with carbon availability (Herrero et al. 2001). The degree of phosphorylation is also expected to be influenced by the energy status of the cell as determined by the balance of ATP and reductant.

Depending on its conformational state, the PII protein interacts with various target proteins, most of which regulate the nitrogen assimilatory pathways (Forchhammer 2008). This includes nitrate/nitrite permeases, bicarbonate uptake and gene expression through the global nitrogen control factor NtcA (Forchhammer 2008). The non-phosphorylated form inhibits nitrate/nitrite uptake while in the phosphorylated form this inhibition is relieved (Tandeau de Marsac et al. 2001).

The nitrogen-control transcription factor NtcA is the major mediator of global nitrogen control at the level of gene expression (Luque et al. 1994; Schwarz and Forchhammer 2005). The activity of NtcA is subject to metabolic regulation, such that under conditions of nitrogen excess (low 2-oxoglutarate levels), the NtcA protein is inactive (Luque et al. 2004) while increased 2-oxoglutarate concentrations and the absence of ammonium lead to stimulation of NtcA activity (Vázquez-Bermúdez et al. 2003; Schwarz and Forchhammer 2005). NtcA regulates the expression of genes encoding for the assimilation of ammonium, or alternative nitrogen sources when cells are incubated under limiting concentrations of ammonium but with adequate carbon to give the cells a high C to N ratio. When NtcA is activated there is a high expression of the *glnB* gene that produces the PII protein. The PII protein that is synthesised, ligands with 2-oxoglutarate which is in high concentrations and ATP. This negatively controls the affinity uptake of bicarbonate and releases inhibition of the nitrate/nitrite transporters. This research has suggested a complex interaction between PII and NtcA where phosphorylated PII activates NtcA and in turn the activated NtcA augments the levels of NtcA and PII as well as stimulating PII phosphorylation (Schwarz and Forchhammer 2005) (Fig. 6.8).

The extent of the link between PII and 2-oxo-glutarate was also found to affect the metabolism of carbon. Whatever its phosphorylated state, PII was found to negatively influence the high affinity uptake system for bicarbonate under high inorganic carbon (*Ci*) conditions when 2-oxo-glutarate presence is elevated (Tandeau de Marsac et al. 2001). This sug-

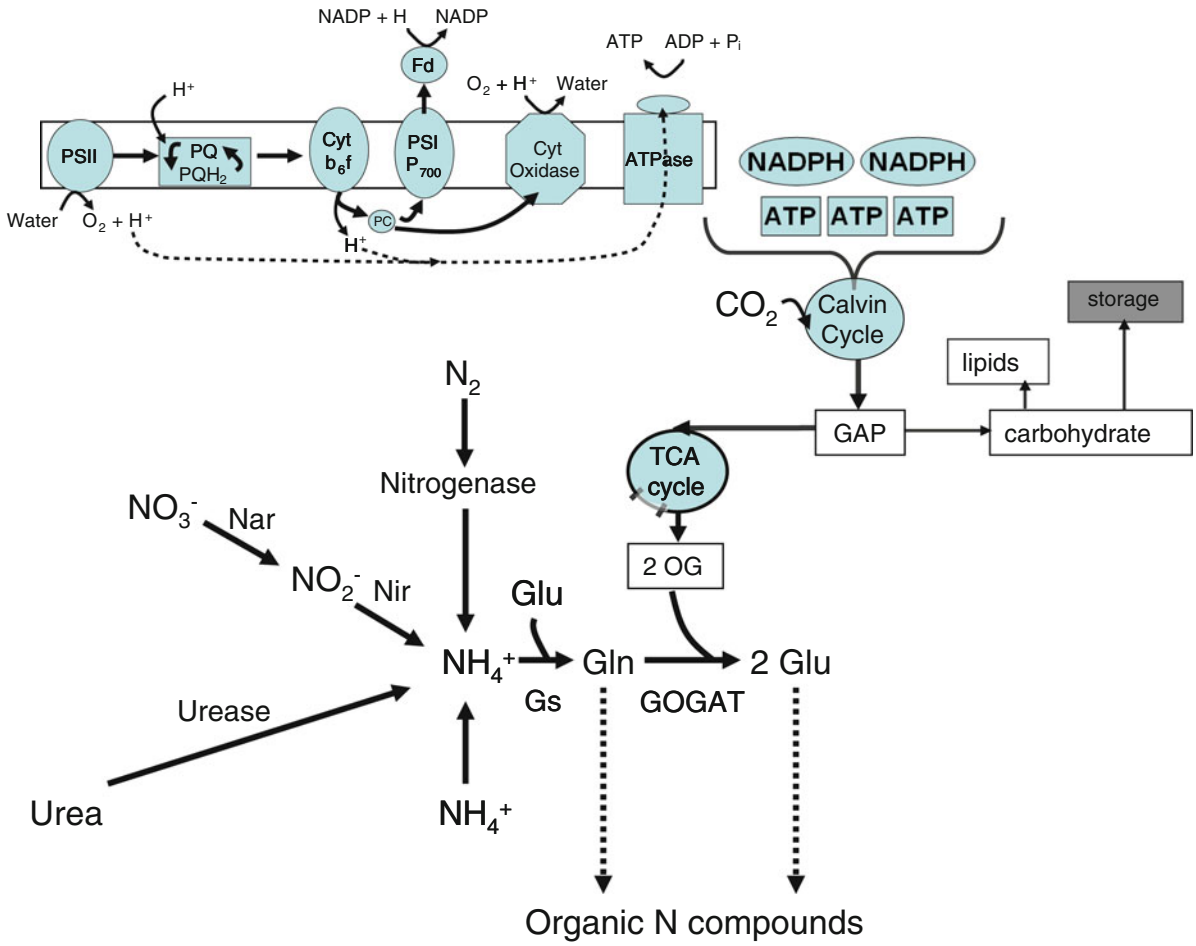


Fig. 6.7 Major nitrogen assimilation pathways in cyanobacteria. Additional symbols: Nitrate reductase (Nar), Nitrite reductase (Nir), Glutamine synthetase (GS), Glutamate synthase (GOGAT), Glutamine (Glm), Glutamate (Glu)

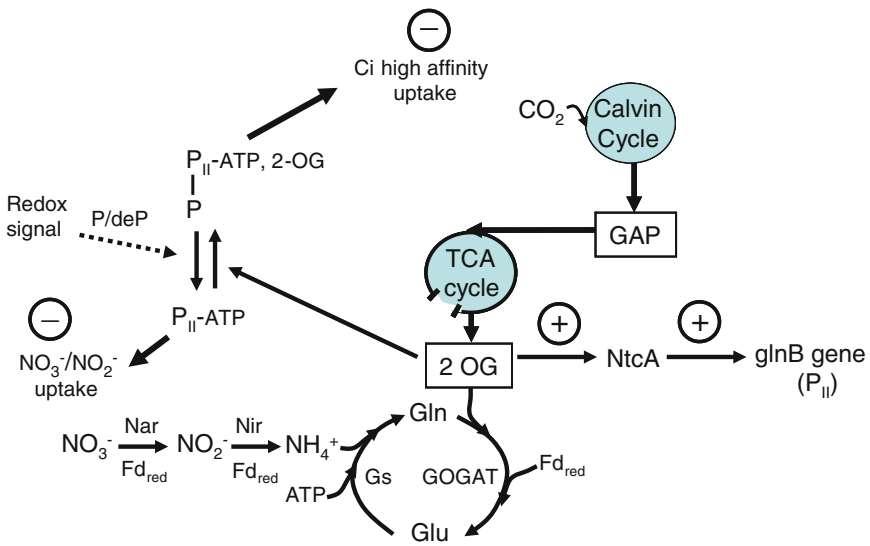


Fig. 6.8 Interactions between nitrogen and carbon metabolism in cyanobacteria with positive and negative influences indicated by plus and minus (Redrawn from Tandeau de Marsac et al. 2001). Additional symbols: Nitrogen-control transcription factor (NtcA), gene encoding the PII protein (glnB), Reduced ferredoxin (Fd_{red}), Inorganic carbon (C_i)

gests that the extent to which PII is linked with 2-oxo-glutarate has opposite effects on the nitrate/nitrite and the high affinity *Ci* uptake system (Fig. 6.8). Such a system would allow an intracellular N/C balance to be maintained in response to changes in the nitrogen and/or carbon regimes of the cells (Tandeau de Marsac et al. 2001).

The key enzyme of the arginine synthesis pathway, N acetylglutamate kinase (NAGK), was also identified to

be a receptor for PII (Heinrich et al. 2004; Burillo et al. 2004; Schwarz and Forchhammer 2005) influencing the degree of cellular nitrogen storage (Heinrich et al. 2004; Maheswaran et al. 2006). Complex formation and catalytic activation of NAGK by PII was shown to depend both on the phosphorylation state of PII and on its binding of effector molecules (Sect. 6.6).

Nitrogen assimilation influences the rate of CO_2 fixation, the fate of newly fixed carbon, and the level of carbohydrate reserves (Guerrero and Lara 1987; Turpin 1991; Garcia-Gonzalez et al. 1992; Tapia et al. 1996) with major effects expected on cell growth, cell turgor pressure and cell density (Sect. 6.11).

6.5 Nitrogen Fixation

When other sources of inorganic nitrogen have become limiting, cyanobacteria equipped with the nitrogenase enzyme complex can utilise N_2 gas through the process of nitrogen fixation. In contrast there are no known micro-algae that can fix molecular nitrogen. This provides the nitrogen fixing cyanobacteria with a major advantage when sources of combined inorganic nitrogen are depleted from the water. However, the fixation of nitrogen requires a substantial amount of energy, using 16 ATP molecules as well as eight electrons to produce two molecules of ammonium and one molecule of H_2, the H_2 being respired under oxygenic conditions (Stal 2009). In addition to this the production and maintenance of the nitrogenase enzyme is a significant energy cost as it comprises about 10% of total cellular protein.

Cyanobacteria are the only nitrogen fixing organisms that also produce oxygen through photosynthesis, and this creates difficulties as the nitrogenase complex is inhibited by oxygen. This has resulted in an array of morphological, metabolic and behavioural adaptations within the different cyanobacteria to protect nitrogenase activity and enable nitrogen fixation (Berman-Frank et al. 2003; Stal 2009). Separation of nitrogen fixation from photosynthesis is achieved either by spatial segregation, temporal separation, or a mixture of both, and to some extent reflects the habitat to which the cyanobacteria are adapted (Berman-Frank et al. 2003). These structural and metabolic patterns were classified into four groups by Berman-Frank et al. (2003) as shown in Fig. 6.9. These range from colony types with nitrogenase distributed evenly across all cells to those with specialised cells called heterocysts that are specifically differentiated for nitrogen fixation. Marked differences occur between taxa in response to diel changes in light flux and there will indeed be many subtle variations of these idealised profiles, particularly given the variability of light flux induced by position in the water column and temporal variation in the light field at sub-daily (e.g. from clouds, wind-waves), daily and seasonal time scales.

In the two groups of cyanobacteria that have nitrogenase activity evenly distributed across cells (eg. *Plectonema*, *Lyngbya*), nitrogen fixation is separated in time from photosynthesis and occurs largely at night, supported by the catabolism of C reserves that were synthesized during the previous light period. In the first of these two groups nitrogen fixation only occurs under microaerobic conditions. This protects nitrogenase from oxygen while still supplying sufficient oxygen to support the production of ATP and reductant through respiration. In the second group nitrogen fixation can occur in oxygenic conditions with peak nitrogenase activity coinciding with high respiration rates 12 h after the peak of photosynthetic activity. In this case the regular pattern continues even under continuous light suggesting circadian control (Berman-Frank et al. 2003).

In the filamentous, non-heterocystous, marine cyanobacterium *Trichodesmium,* there is a complex interaction between spatial and temporal segregation of the photosynthetic, respiratory and nitrogen fixing activities. Measurements of photosynthesis and nitrogen fixation show a temporal separation during the photoperiod with photosynthesis peaking in the morning and afternoon offset by ca. 6 h from the peak of nitrogen fixation. During the night nitrogenase is inactivated and turned over under the control of a circadian clock (Berman-Frank et al. 2007). Activation of nitrogenase in cells is linked to a reduction in gross photosynthesis and increased respiratory activity that results in a negative net production of oxygen which creates the conditions suitable for nitrogenase activity. There is no evidence that any cells undergo unidirectional differentiation as occurs in the formation of heterocysts within some filamentous cyanobacteria (Stal 2009). Active photosynthetic components are found in all cells but it is uncertain whether all cells have the capacity for nitrogen fixation, or whether nitrogenase occurs in all cells or in a fraction of cells arranged consecutively along the trichome (Berman-Frank et al. 2007; Stal 2009). Whatever the arrangement, nitrogen fixation involves cells switching between photosynthesis and nitrogen activity to give a combined temporal and spatial response. This mode of nitrogen fixation has not been reported for freshwater species.

In some filamentous cyanobacteria, N_2 fixation takes place in specialized cells called heterocysts that differentiate irreversibly from vegetative cells 12–20 h after combined nitrogen sources are removed from the medium. This occurs when ambient nitrate concentrations are depleted to ca. 0.3–1.6 μmol L^{-1} but there is considerable variation amongst different cyanobacteria in the duration of persistence of heterocysts when nitrate concentrations return to levels exceeding this range (Holl and Montoya 2005; Agawin et al. 2007). Maximum in situ abundance of heterocysts amongst vegetative cells therefore appears to be closely aligned with very low concentrations of dissolved inorganic nitrogen but has been shown by Wood et al. (2010) to precede

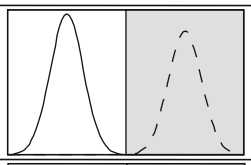
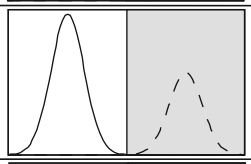
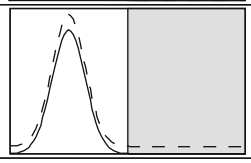
Class	Morphology: nitrogenase	Examples	Nitrogen fixation
Microaerobic Type I	Filaments: Evenly distributed	*Plectonema*	
Aerobic Type II	Filaments or unicells: Evenly distributed	*Cyanothece* *Lyngbya*	
Some cellular differentiation Type III	Filaments: Specialised or localised cells	*Trichodesmium* *Katagnymene*	
Cellular differentiation Type IV	Filaments: Heterocysts	*Anabaena* *Nostoc*	

Fig. 6.9 Classes of cyanobacteria derived by Berman-Frank et al. (2003), based on morphology, location of nitrogenase activity and behavioural adaptations enabling nitrogen fixation. The graphs show idealised representation of day (unshaded) and night (shaded) periods and rates of photosynthesis (solid lines) and nitrogen fixation (dashed lines)

the peak in vegetative cell biomass often associated with blooms of *Anabaena planktonica*. Heterocyst differentiation is strictly controlled by the nitrogen transcription factor NtcA linking differentiation to nitrogen deficiency (Flores and Herrero 2005). The heterocysts have a modified metabolism to maintain a microaerobic environment for nitrogenase expression while providing ATP and electrons for nitrogenase function. The heterocysts do not contain PSII and so do not generate O_2, but consequently cannot fix CO_2. Instead they rely on a supply of fixed carbon from adjacent cells for respiratory substrate to provide reducing equivalents, vegetative cells in return receiving fixed nitrogen from the heterocysts. The heterocysts generate ATP in the light by cyclic electron flow around PSI but the extent of this depends on the light conditions (Berman-Frank et al. 2007; Stal 2009). In the dark, respiration is the only source of energy.

Heterocystous cyanobacteria fix N_2 during the day in parallel with photosynthesis in the vegetative cells. In some cases nitrogen fixation continues into the night and this continued activity is supported by the transfer of fixed carbon from stores accumulated in the vegetative cells. However, at non-saturating light intensities and in the dark, nitrogenase activity can become energy limited (Stal 2009). In order to optimise energy generation the O_2 influx into the heterocysts needs to be as large as possible, but without exceeding the respiration capacity, so that anoxic conditions can be maintained. As the cyanobacteria occur in dynamic environments they need to respond quickly to changes in conditions in order to perform optimally. The heterocysts have a thick cell wall that carries extra glycolipid and polysaccharide layers to reduce gas permeability (Walsby 1985), but this influences both O_2 and N_2 fluxes and is unlikely to change quickly (Stal 2009). It has been suggested (Walsby 2007) that the diffusion properties of the cell wall may slowly respond to growth conditions, and that dynamic gas exchange is controlled by the pores that connect the heterocyst with neighbouring vegetative cells.

N_2 fixation confers a significant competitive advantage for cyanobacteria but its contribution to nitrogen inputs at a system scale may vary. *Nodularia spumigena* contributed more than 81% of the annual N inputs to a high altitude hyposaline lake in the United States (Horne and Galat 1985). Schindler et al. (2008) generalised that N inputs from fixation could overcome deficiencies of N in lake phytoplankton assemblages and advocated a phosphorus control paradigm to manage cyanobacterial blooms. Scott and McCarthy (2010) subsequently refuted the capacity for N fixation to provide for N replete conditions in Lake 227, Canada, when they showed that phytoplankton biomass decreased in response to a reduction in total nitrogen concentration. Translating conceptual models of N-fixation (e.g., Oliver and Ganf 2000) into numerical formulations is challenged by the temporal and spatial variability of N-fixation. Levine and Lewis (1987) developed a model of N-fixation by heterocystous

cyanobacteria for Lake Valencia, Venezuela, to estimate the importance of fixation for lake N inputs. Their model is closely linked to rates of photosynthesis to reflect a baseline rate of N fixation at night which is ramped up with photosynthesis during the day:

$$N_{fh} = N_s(1-e^{-a})e^{-b} + D$$

where N_s is the maximum rate of N_2 fixation per heterocyst in the absence of light, $a = \alpha E N_s^{-1}$ and $b = \beta E N_s^{-1}$ where I is light intensity, α is a parameter for the slope of the rising limb of the light response curve and β is a photoinhibition parameter, and D is the rate of N_2 fixation per heterocyst in the dark. Values for N_s given by Levine and Lewis (1987) are up to a maximum of nearly 0.04 µmol $(10^6$ heterocyst$)^{-1}$ h^{-1} which compares with a value of 7×10^{-13} g N heterocyst^{-1} h^{-1} (or 0.05 µmol $(10^6$ heterocyst$)^{-1}$ h^{-1}) given by Howarth et al. (1993). For more general models of ecosystem processes that include cyanobacteria, Howarth et al. (1999) present the first known model that differentiates N fixing from non-N fixing cyanobacteria. They use a multiplier term (N_{mult}) to enhance growth rates under low DIN concentrations:

$$N_{mult} = \frac{\text{DIN}}{\text{DIN} + Km_N}$$

where Km_N is the half-saturation constant for uptake of DIN, taken to be 20 µM by Howarth et al. (1999) (see also Zevenboom and Mur 1978). The corresponding N uptake from fixation is assumed to meet all of the N demand for growth and there is no DIN assimilation from the water column. Another multiplier term (Mo_{mult}) is used to account for the availability of molybdenum (Mo):

$$Mo_{mult} = 4.2\frac{\text{Mo}}{\text{Mo} + Km_{Mo}\left(1+\frac{\text{S}}{Ki}\right)}$$

where Km_{Mo} is the half-saturation constant for uptake of Mo, Ki is an inhibition constant for the effect of sulphate (S) on Mo uptake and 4.2 is a scaling factor where Mo_{mult} is assigned a value of one for concentrations of Mo and S encountered in freshwaters. Few ecosystem models deal with potential for micronutrient limitation or explicitly include micronutrients as state variables, but limitation by iron or other trace elements such as molybdenum may potentially be alleviated by periods when these micronutrients are present in high concentrations (Donnelly et al. 1997), even in freshwater systems where their chelation by salts is greatly reduced compared with estuarine or marine systems.

The N derived from fixation is assumed in the Howarth et al. (1999) model to be in direct proportion to P assimilated, at a molar ratio of 15:1, to maintain constant nitrogen content per cell of 3.3×10^{-12} mol N.

An alternative approach to modelling N_2 fixation has been used by Stal and Walsby (1998) in which the rate of fixation N_{fc} is expressed as a chlorophyll-specific value:

$$N_{fc} = N_{sc}(1-e^{-c}) + D + d$$

where N_{sc} is the maximum rate of N fixation at light saturation, D is the rate of N fixation in darkness, $c = -\alpha E / N_{sc}$ and d (negative) is a phtoinhibition parameter ($d = \beta E^2$). This formulation is similar to that used by Levine and Lewis (1987) except for an expression for the effects of photosynthesis on N fixation. Stal and Walsby (1998) used values for N_{sc} of 6.5 µmol N_2 (mg chl-a)$^{-1}$ h^{-1} and D of 1.48 µmol N_2 (mg chl-a)$^{-1}$ h^{-1}. Other values of N_{sc} include up to 6.48 µmol N_2 (mg chl a)$^{-1}$ h^{-1} (Ohlendiek et al. 2000), and 4.59, 1.34–4.64 and 6.5 for *Aphanizomenon* sp., *Nodularia* spp. and a Baltic Sea sample (Stal and Walsby 2000). A conversion between these values and those given above by Levine and Lewis (1987) and Howarth et al. (1993) would require several assumptions including the ratio of heterocysts amongst the vegetative cells, considered by Levine and Lewis (1987) to be rarely more than 1:10, as a chlorophyll specific mass per vegetative cell.

A different approach is used by Hense and Beckmann (2006), recognising a threefold increase in energy requirements for growth using fixed N, to reflect the utilisation of at least 16 ATP molecules per N_2 molecule reduced. According to their model the heterocystous form of a four-stage cyanobacterial life cycle occurs when there is low DIN, but adequate energy reserves in the cell. The fixation rate is given by

$$N_{fx} = \omega_{fx}\sigma_E C$$

where N_{fx} represents the accumulation rate of organic nitrogen in the cell as a result of fixation, ω_{fx} is the growth rate defined in terms of change in nitrogen, σ_E is a limitation function for the internal energy store, represented by:

$$\sigma_E = 1 - \left(\frac{En}{En_{\max}}\right)^n$$

Where En and En_{max} are equivalent to internal energy storages and n is an assigned exponent. In this model an internal energy quota regulates partitioning between growth (when the internal N quota is large) and uptake (as the quota decreases). Hense and Burchard (2009) analysed the sensitivity of their four-compartment cyanobacterial life cycle model, which included akinetes, recently germinated vegetative cells, and heterocystous and non-heterocystous stages,

against simpler alternative models. They showed a 30% variation in simulated annual N_2 fixation rates for the dominant diazotroph, *Nodularia spumigena*, in the Baltic Sea. The model of Hense and Burchard (2009) is one of the few to include a benthic stage (akinetes), but in general mechanistic modelling of overwintering (e.g. in *Microcystis* – see Chap. 7) has either been ignored or is implicitly included in the parameterisation of planktonic processes.

6.6 Nitrogen Storage

Cyanobacteria have the capacity to store significant amounts of N in excess of their immediate requirements. The two storage components are phycocyanin, a phycobiliprotein, and cyanophycin, a co-polymer of aspartate and arginine. Whereas phycocyanin is also a major pigment component of the light harvesting antenna, the primary function of cyanophycin is to store nitrogen and perhaps energy (Allen 1984; Kolodny et al. 2006). Cyanophycin and phycocyanin are both at low concentrations in N-limited cells. Cyanophycin occurs in low concentrations during balanced growth of non-diazotrophic cyanobacteria, but it can occur in large concentrations in diazotrophic cyanobacteria and acts as a transient store for newly fixed nitrogen in heterocysts. Cyanophycin accumulates in cyanobacteria when they are grown under all unbalanced nutrient conditions except nitrogen starvation, and it is used as a nitrogen source before other sources during N-starvation (Kolodny et al. 2006). In a manner reminiscent of the P luxury storage phenomenon, cyanophycin accumulates on the addition of a useable nitrogen source to N-limited cells (Simon 1987). Cyanophycin synthesis peaks sometime after the addition of N and then decreases to the levels found in typical exponentially growing cells (Mackerras et al. 1990a, b; Kolodny et al. 2006). In response to nitrogen starvation the cyanophycin granules are first degraded, followed by cell bleaching due to degradation of components of the phycobilisome including phycocyanin (Tandeau de Marsac and Houmard 1993). Nitrogen stores are also utilised when low-light cells are shifted to high light, with cyanophycin and phycocyanin both decreasing.

Control of cyanophycin formation is through the enzyme for arginine biosynthesis, N-acetylglutamate kinase (NAGK). NAGK is linked to cell N status through the signalling protein PII and its interaction with 2-oxo-glutarate and ATP/ADP (Llácer et al. 2008). NAGK activity is strongly enhanced by complex formation with the non-phosphorylated form of PII that is produced when N is abundant and 2-oxo-glutarate levels are reduced. This binding also releases NAGK from arginine feedback inhibition. Under these conditions high levels of arginine build up and cyanophycin synthetase can produce cyanophycin stores (Flores and Herrero 2005; Maheswaran et al. 2006; Llácer et al. 2008). When nitrogen is scarce, 2-oxo-glutarate accumulates and binds to PII in the presence of ATP and this promotes dissociation of the PII-NAGK complex decreasing activity and enabling arginine inhibition. Under these conditions arginine levels are below those required for cyanophycin formation by cyanophycin synthetase (Maheswaran et al. 2006).

6.6.1 Nitrogen Starvation

Prolonged nitrogen starvation causes a series of cellular changes in cyanobacteria. Firstly, a rapid degradation of phycobilisomes occurs before other proteins and pigments are utilized. The cells finally become almost completely depigmented and enter a survival mode (Schwarz and Forchhammer 2005). These changes are reversible, and following the addition of a combined nitrogen source the cells return to vegetative growth within a few days. Sauer et al. (2001) analysed *Synechococcus* cells that were kept in nitrogen depleted conditions for more than 2 months. The cells retained residual PSI and PSII activity at about 0.1% of growing cells. Using protein labelling techniques it was shown that the apparently dormant cells turned over proteins associated with photosynthesis and redox homeostatis, but not proteins involved in the translational machinery.

6.7 Cellular Elemental Stoichiometry

The Redfield ratio of 106C:16N:1P is the average cellular mole ratio of carbon, nitrogen and phosphorus originally derived from measurements of marine phytoplankton. It has been used widely in aquatic studies to determine the nutrient status of systems, to link biogeochemical models of these elements, and to estimate cellular production (Geider and La Roche 2002). For example, the N:P ratio of 16:1 is frequently used to identify whether systems are more likely to be phosphorus or nitrogen limited assuming that N:P < 16 is indicative of N limited conditions. The reliability of these assumptions has been questioned frequently in view of the large elemental fluctuations that have been observed in phytoplankton (Hecky et al. 1993). Geider and La Roche (2002) reviewed the data on C:N:P ratios in cultures grown under nutrient replete, nutrient limited and optimal growth conditions. They also estimated elemental ratios based on the likely biochemical composition of physiologically competent cells in order to constrain the elemental ranges measured in cultures. They concluded that the laboratory data do not support the idea of a biochemically fixed C:N:P ratio in the proportions defined as the Redfield ratio. They found the N:P mole ratio in cultures to range from <5 to >100. Even under optimal growth conditions the range was from 5 to 19 N:P with most measurements below the Redfield ratio.

Biochemical calculations suggested likely N:P compositions between 15 and 30. The transition between N and P limitation was estimated from limited data to be more likely in the range of 20–50 N:P, substantially higher than the Redfield value of 16.

In contrast, the C:N mole ratio, although still variable, was much more constrained, especially in optimally growing, nutrient-replete cultures where it was on average close to the Redfield ratio of 6.6. These observations support findings from integrated studies of nitrogen assimilation and carbon fixation suggesting that a range of mechanisms appear to be targeted at maintaining the cellular C:N ratio within narrow bounds during growth.

The variability in ratios, particularly of N:P and C:P, reflects the known physiological plasticity of phytoplankton and also the phenomenon of luxury consumption where non-limiting nutrients can form intra-cellular stores. Cyanobacteria store nitrogen as cyanophycin when phosphorus and other growth requirements are limiting, and store phosphorus as polyphosphate when nitrogen and other growth requirements are limiting. Phosphorus storage as polyphosphate markedly reduces the N:P and C:P ratios so broadening the range of possible values. In contrast, nitrogen storage as cyanophycin has a N:C mass ratio of ca. 0.5, which is not greatly different from the typical values observed in cells, so it has a lesser effect on overall cellular N:C stoichiometry. Carbohydrate accumulation under nutrient-limited conditions will also influence the carbon ratios.

6.8 Whole Lake Nutrient Influences

The classical work of a number of authors in the 1960s (e.g. Sakamoto 1966; Vollenweider 1968) led to the recognition of the importance of increased phosphorus loadings in the process of eutrophication of lakes. These studies on phosphorus, and later studies on the interaction between nitrogen and phosphorus (e.g. Smith 1983), led to ecological research focused on the manipulation of whole lakes or portions of them to explore the responses of phytoplankton abundance and community structure to nutrient conditions (Schindler 1971; Lund and Reynolds 1982). Many of these studies have focused on total nutrients rather than bioavailable forms. The bioavailable forms are commonly considered to be in dissolved inorganic form but in some mesotrophic lakes dissolved organic nutrients (e.g. for *Aphanizomenon flos-aquae* in Lake Kinneret) can also constitute an important component of the cellular nutrient uptake, particularly in the presence of alkaline phosphatise to mobilise organic phosphorus (Berman 1997).

On the basis of the Redfield ratio, and the fact that many of the bloom-forming cyanobacteria can fix nitrogen, it was suggested that cyanobacteria should dominate in waters with low N:P ratios (Smith 1983; Downing et al. 2001). This was supported by an analysis of 20 lakes largely from northern Europe (Smith 1983), but the conclusion was questioned because some samples were not considered to be independent or representative of the lakes. Further analysis of these data by Trimbee and Prepas (1987) suggested that the individual nutrient concentrations, either total phosphorus or total nitrogen, provided more reliable estimates of average cyanobacterial dominance than their ratio. Similarly, Downing et al. (2001) carefully collected and prepared information from published reports to create a dataset of 269 observations from 99 lakes around the world. This was used to investigate further the influence of nutrient stoichiometry (i.e. N:P ratio) on cyanobacterial dominance, compared to nutrient concentrations (N or P), or algal biomass. They concluded that the risk of water quality degradation by cyanobacteria blooms was more strongly correlated with variation in total P, total N, or standing algal biomass than the ratio of N:P. They suggested that correlations between N:P and cyanobacterial dominance were the result of a strong negative correlation between nutrient enrichment and the N:P ratio due to nutrient sources often being depleted in N relative to P (Downing et al. 2001). These findings are in accord with doubts about the reliability of the Redfield ratio for drawing conclusions about system scale responses.

These outcomes are important to water quality management as the notion that a TN:TP molar ratio above c. 15 indicates a switch from a high to a low potential for cyanobacterial dominance has been misinterpreted with major implications for catchment scale nutrient management. Schindler et al. (2008) were concerned that many studies in lakes and estuaries were still concluding that N must be controlled as well as, or instead of, P to reduce eutrophication. They described a 37-year whole lake experiment testing the effect of N reduction and concluded that to reduce eutrophication the focus of management must be on decreasing P inputs. Downing et al. (2001) concluded from their analyses that the most potentially useful of the relationships was that based on total P, which predicts phytoplankton biomass and clearly discriminates lakes dominated by cyanobacteria. They suggest that the risk of dominance by cyanobacteria is only 0–10% between 0 and 30 μg L^{-1} total P, rising abruptly to about 40% between 30 and 70 μg L^{-1}, and reaching an asymptote at around 80% near 100 μg L^{-1}. This does not mean that management of N enrichment should be ignored, but that the principle focus should be on phosphorus control (Carpenter 2008).

The relationship between eutrophication and an increased biomass of gas-vacuolate cyanobacteria has been attributed to the requirement that sufficient nutrients need to be available, either in the water or from internal recycling, when physical conditions eventually become suitable to provide the cyanobacteria with a competitive advantage. In temperate systems, if nutrients are depleted by phytoplankton growth during

spring and early summer, then the bloom-forming cyanobacteria are faced with depauperate nutrient conditions when the physical environment is most suitable for their growth. Similar arguments can be made for tropical waters, but on cycles driven by daily to weekly meteorological events as well as seasonal conditions (Lewis 1978a, b). It is under these conditions that resting stages such as akinetes or an overwintering phase (*Microcystis*; Chap. 7) can be highly advantageous and allow for rapid recolonisation of the water column when conditions become favourable again.

6.9 Modelling Nutrient-Dependent Growth Rates

Flynn (2003b) identified three modelling strategies to describe nutrient uptake by phytoplankton. In order of increasing complexity these are the Monod, quota and mechanistic modelling approaches. The Monod model makes growth rate a direct function of the external nutrient concentration. The quota model makes the internal nutrient content (the quota) the controlling factor, although the quota is itself a function of the external nutrient supply. The more complex mechanistic models, with feedback processes and perhaps multiple internal pools, seek to simulate more closely biochemical reality (Baird and Emsley 1999; Flynn 2003b).

The Monod model relates the nutrient specific steady-state growth rate (μ_i) to the external concentration of the nutrient (x_i) and a half saturation constant for growth (k_i) according to a rectangular hyperbolic function:

$$\mu_i = \frac{x_i}{x_i + k_i}$$

When multiple nutrients are being considered a threshold approach is generally used with the nutrient giving the slowest growth rate considered to be controlling. This is often determined according to the Redfield ratio. Once a nutrient is selected as limiting then others are ignored, or assumed to respond in a fixed proportion which is again frequently set by the Redfield ratio. This type of modelling approach cannot account for alterations in internal nutrient reserves or the changes in nutrient ratios that occur when cells modify their responses to changing environmental conditions.

In comparison the quota model (Droop 1968) relates growth to the internal concentration of a nutrient, which means that growth can continue at the expense of previously accumulated nutrient stores. In the quota model the nutrients can be described in relation to cellular carbon levels so that the model can also simulate carbon biomass or it can be formulated on a cellular basis. One form of the model is that described by Caperon and Meyer (1972):

$$\mu_c = \mu_{mX} \frac{X_c - X_{co}}{X_c - X_{co} + k_{qX}}$$

Where μ_c is the carbon-specific growth rate, X_c the carbon based nutrient quota (nutrient:C ratio), X_{c0} the minimum carbon based cell quota of the nutrient at which growth is zero, k_{qX} is a curve fitting constant similar to the half saturation constant, and μ_{mX} the maximum theoretical growth rate when using nutrient X. As with the Monod equation, the usual method to address multiple nutrient influences has been the threshold approach, although multiplicative interactions have been investigated. Neither the multiplicative or threshold formulations have a firm mechanistic basis.

Both the Monod and quota models are only valid under conditions of balanced growth, when the specific rates of change of all cellular components are equal. This occurs when nutrient uptake, light harvesting and carbon fixation are directly coupled giving a fixed cell stoichiometry. Consequently these models are restrictive when modelling multi-nutrient interactions, or light nutrient interactions. The models cannot describe the uncoupled rates of nutrient assimilation or carbon fixation that occur in response to rapid fluctuations in environmental conditions (Geider et al. 1998). As the elemental composition of phytoplankton does not necessarily conform to the Redfield ratio (Geider and La Roche 2002), it is no longer acceptable to assume that knowledge of one element enables the concentration of other elements to be calculated (Baklouti et al. 2006). As a result it has become necessary to develop multi-nutrient models and to explore the possibilities of multi-nutrient limitation and co-limitation.

6.10 Modelling Metabolic Interactions and Cellular Growth

Whereas many models disaggregate the functions of photosynthesis, nutrient uptake and cellular composition, it is apparent that these are closely linked, and a major improvement in modelling has come from the consideration of integrated energy and material fluxes through the cellular machinery (Shuter 1979; Behrenfeld et al. 2008; Singh et al. 2008). This considers that various cellular mechanisms are involved in trying to maximise cellular growth through linked homeostatic interactions (Behrenfeld et al. 2008). It is suggested that the pigment concentration is adjusted to optimise delivery of ATP and reductant under the available light conditions, but modified in response to the overall cellular demand created by the particular growth environment. The required supply of ATP and reductant is influenced by the balance between the demand for carbon fixation to provide new carbon components in metabolic pathways, the necessity to support other cellular metabolic activities directly from

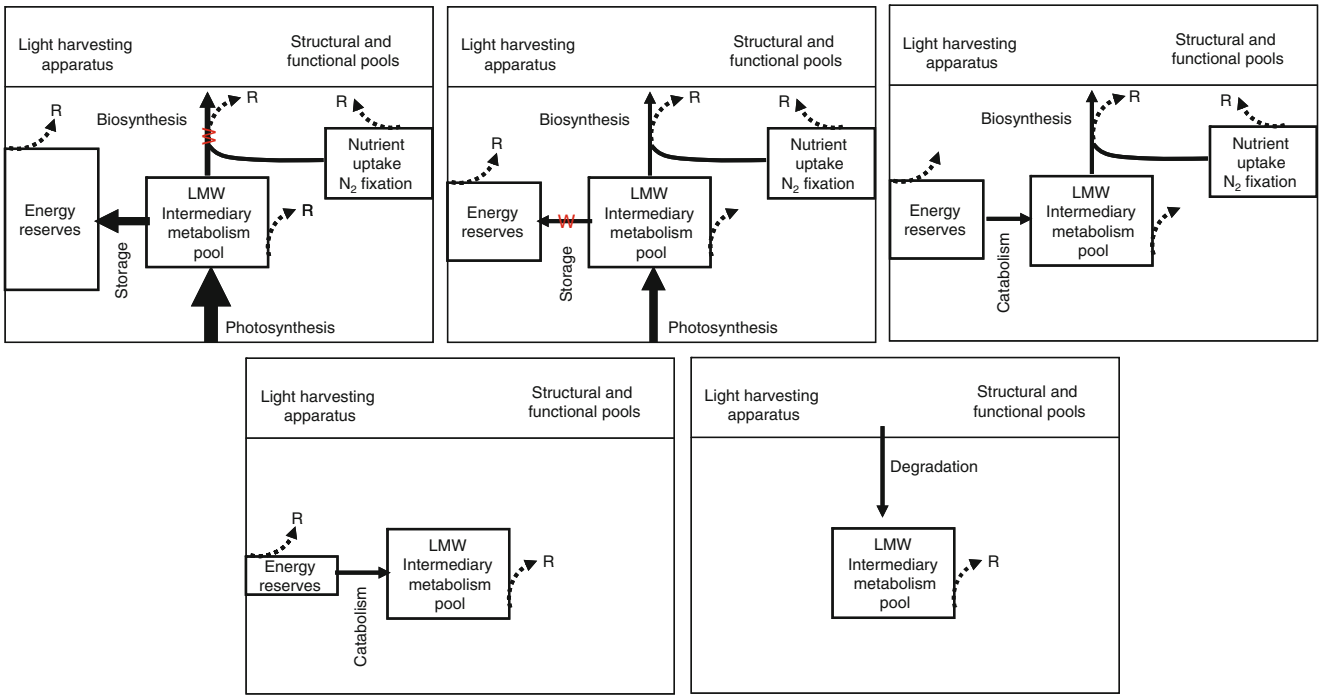


Fig. 6.10 Major cellular components and fluxes that have been included in compartment models to describe fluctuations in cellular composition during unbalanced growth. The series of figures depict the effects of decreasing light intensity followed by increasing periods of dark. Respiration (R), Low molecular weight carbohydrates (LMW), resistance to pathway flux (W)

the photosystem, and the requirement for storage products. These alternatives are influenced by alterations in the dominating metabolic pathways and their ATP and reductant requirements. There is a constant adjustment to minimise differences in the production and demand ratios for ATP and reductant (Behrenfeld et al. 2008). Such physiologically based models that describe cell function can include feedback mechanisms that more closely reflect reality.

If the variability in cell stoichiometry is considered to be largely due to stored nutrients while the structural components remain relatively constant (Klausmeier et al. 2004; Baklouti et al. 2006), then distinguishing between cellular pools is important. This concept identifies the need for more physiological modelling to describe these components pools. This approach has been explored in several models (Shuter 1979; Geider et al. 1996; Rabouille et al. 2006; Ross and Geider 2009) and appears to be promising (Fig. 6.10).

In the various cellular compartment models the components have been identified in different ways depending on the purpose of the model. Rabouille et al. (2006) in developing a physiological growth model to describe nitrogen fixation in *Trichodesmium* recognised four compartments. Cellular carbon was divided into three pools; the low molecular weight carbohydrates from photosynthesis, the internal carbon reserves, and the structural biomass. In this formulation, the low molecular weight pool has a short turnover time and is the intermediate for all metabolic processes. It is consumed either to build carbon reserves, or for incorporation into structural components, or to supply energy through respiration for maintenance and biosynthesis. The low molecular weight pool is supplied by photosynthesis or by catabolism of carbohydrate reserves. The presence of a carbohydrate reserve enables a cell to continue biosynthesis for some time into the night using carbon fixed during the day. In this model cellular nitrogen was also described in terms of an internal storage pool that grouped glutamine and the nitrogen reserve components cyanophycin and phycobiliproteins and was fed by the uptake of external nitrogen. This provided the intracellular nitrogen pool available for the synthesis of structural biomass through biosynthesis with carbon (Fig. 6.8). By incorporating two separate reserves of carbon and nitrogen with unbalanced dynamics the fluctuations in cellular composition could be investigated and the effects of these on nitrogen fixation assessed (Rabouille et al. 2006).

Ross and Geider (2009) used a similar but simpler approach to model photosynthesis and photoacclimation under nutrient replete conditions. Their model contains only two carbon pools, a functional and a reserve pool. Their model alludes to, but does not describe, the dynamics of a low molecular weight component. The Ross and Geider (2009) model has nitrogen in the functional pool occurring at a fixed ratio with carbon and alterations in the cellular N:C ratio are attributed

to the accumulation or catabolism of carbohydrate reserves. This model also incorporates consideration of the light harvesting apparatus so that photosynthesis and photoacclimation can be included.

These cellular compartment models are in their infancy, but provide a framework for integrating the new molecular and biochemical information that more clearly describes the interactions in cellular photochemistry and metabolism that operate to maximise cell growth while minimising damage. The models have further attributes that will be useful in considering the growth and ecology of cyanobacteria as well as phytoplankton generally. They are likely to be of particular value to the modelling of planktonic cyanobacteria because of: (i) the roles that different forms of carbohydrate play in buoyancy regulation and the interactions with nutrients and light (Oliver 1990; Oliver and Ganf 2000); (ii) the cellular differentiation, often associated with nitrogen fixation, that requires individual cell conditions to be modelled within the context of colonial responses (Adams and Duggan 1999); (iii) the complex lifestyles of different groups that can include resting stages that overwinter in the sediments (Hense and Beckmann 2006). As they are cell based the physiological functions can be connected with cell size and geometry, a powerful link that has been used in empirical models simulating light-nutrient interactions (Reynolds et al. 2001). This approach can be taken further with a more theoretical consideration of the geometrically dependent physical processes influencing cellular functions as in the biomechanical approach described in Baird and Emsley (1999).

6.11 Buoyancy and Its Regulation

The success of gas-vacuolate cyanobacteria is often attributed to their ability to regulate buoyancy in response to changing environmental conditions (Reynolds and Walsby 1975; Ganf and Oliver 1982; van Rijn and Shilo 1985; Walsby 1987, 1994; Reynolds et al. 1987). Buoyancy and its regulation confers a competitive advantage for cyanobacteria over the sedimentation losses of many other negatively buoyant phytoplankton (Reynolds 1984), increased access to irradiance (Humphries and Lyne 1988; Walsby et al. 1997), and an ability to bridge the vertical separation that develops in stratified waters between higher availability of nutrients at depth and greater illumination in surface layers (Ganf and Oliver 1982).

6.11.1 Gas Vacuole Structure

Gas vacuoles are comprised of numerous cylinders known as gas vesicles (Bowen and Jensen 1965). These structures are hollow but rigid, proteinaceous cylinders capped at either end by a cone. Specific gas vacuole genes encode for the various proteins used in their synthesis (Walsby 1994; Oliver 1994). Gas vesicles are permeable to gas and therefore contain air of composition similar to that of the surrounding liquid, but the air is not required to maintain the hollow space as the gas vesicle walls are rigid. However, the gas vesicle wall is impermeable to water due to the presence of hydrophobic amino acids on the inner side.

Gas vesicles are ordered into gas vacuoles to occupy minimal space and provide maximum buoyancy. The cylindrical gas vesicles are stacked in hexagonal arrays with the cones interdigitating. In *Anabaena* where the gas vesicle density is 120 kg m^{-3}, if the intervening space (15%) is filled with water at a density of 1,000 kg m^{-3}, then the overall gas vacuole density will be 252 kg m^{-3}, one-fourth the density of water and an efficient mechanism to provide lift (Walsby 1994).

6.11.2 Pressures Acting on Gas Vesicles

The wall of gas vesicles is subjected to hydrostatic and turgor pressures. If the combination of these pressures exceeds the strength of the wall then the gas vesicle will collapse and the cell will lose buoyancy (Walsby 1971). Hydrostatic pressure increases at a rate of 0.1 MPa per 10 m water depth. Episodic deep mixing can potentially entrain cells to greater depths where, in order to retain buoyancy, these cells will need to have gas vesicle walls with sufficient strength to withstand the increase in hydrostatic pressure. Increases in turgor pressure result from increased levels of soluble organic intermediates from photosynthesis (Grant and Walsby 1977), coupled with light-dependent uptake of potassium salts (Allison and Walsby 1981).

Turgor pressure can be measured as the difference in the critical applied pressure (ρ_c) required to collapse vesicles of cells suspended in 0.5 M sucrose which removes the turgor pressure, compared with the pressure (ρ_a) required for turgid cells suspended in filtered lake water or culture medium: $\rho_t = \rho_c - \rho_a$. Turgor pressures can vary from 0 to 0.5 MPa in different organisms (Walsby 1994) and increases can lead to gas vesicle collapse.

The collapse of gas vesicles is dependent on their strength, which may be described by the pressure exerted on them (Walsby 1994):

$$\rho_c = 275(r)^{-1.67}$$

where ρ_c is the critical collapse-pressure (MPa) and r is the cylinder radius (nm). The balance between efficient provision of buoyancy and capacity to withstand hydrostatic pressures appears to be the basis for variations in r amongst species (Hayes and Walsby 1986; Walsby and Bleything 1988; Brookes et al. 1994).

6.11.3 Buoyancy Regulation

Buoyancy regulation occurs in cyanobacteria in response to different environmental stimuli and in turn, allows cyanobacteria to regulate these environmental gradients through vertical movement in the water column (Reynolds and Walsby 1975; Walsby and Reynolds 1980; Reynolds 1987; Oliver 1994; Walsby 1994; Wallace and Hamilton 1999). Buoyancy can be adjusted through changes in the extent of gas vacuolation or by the balance of cellular constituents of different density such as carbohydrates (density, $\rho \sim 1{,}600$ kg m^{-3}) and proteins ($\rho \sim 1{,}300$ kg m^{-3}). Metabolic processes that rapidly alter the size of carbohydrate reserves will be of major significance to buoyancy regulating cyanobacteria as these reserves provide ballast to offset the lift due to gas vesicles. The accumulation of cellular carbohydrate reserves through photosynthesis, or depletion through respiration and conversion to less dense constituents, is one of the most important short-term influences on buoyancy. Changes in other cellular constituents of varying density, including storage materials such as polyphosphate granules, can also affect buoyancy (Romans et al. 1994).

Gas vacuolation can play an important role in buoyancy regulation through changing the extent to which gas vesicles counteract the density of other cellular constituents. Gas vacuolation may be altered through increased turgor pressure acting to collapse gas vesicles, or through the diluting effect of growth and cellular replication (Oliver 1994; Walsby 1994).

N limitation can affect gas vesicle assembly by restricting the production of essential proteins resulting in a loss of buoyancy (Klemer et al. 1982; Chu et al. 2007). The results of laboratory studies (Turpin 1991; Garcia-Gonzalez et al. 1992; Tapia et al. 1996) suggest that where cyanobacteria move between the well illuminated, nutrient-poor surface layers and nutrient-rich aphotic zones, the source of available nitrogen at depth can have a significant effect on rates of buoyancy regulation. For example, the increased availability of ammonium common in the hypolimnion of stratified lakes may cause a reduction in the carbohydrate reserves of sedimenting cyanobacteria leading to a quicker reversal of cell buoyancy and a reduction in the extent of vertical migration. Detailed studies of this interaction are required.

The interplay of limitation by light and macronutrients strongly regulates buoyancy regulation (Konopka et al. 1987b). For example, the accumulation of carbohydrate as polysaccharide occurs in response to excess photosynthate production (Gibson 1978; Kromkamp and Mur 1984), leading to sinking. By contrast carbon limitation leading to depletion of carbohydrate reserves may lead to periods of buoyancy though prolonged carbon limitation and will result in a reduction in buoyancy with reductions in gas vesicle synthesis. This balance between carbohydrate accumulation and incorporation into other compounds is regulated by light availability and cellular nutrient status (Healey 1978).

When light and nutrient supply rates are balanced then the relative growth rate given by μ/μ_{max} is high even if the specific growth rate is low. Under these conditions carbohydrate use is optimised for growth and storage is reduced. If growth is restricted by a limiting nutrient then energy capture exceeds utilisation and carbohydrate is stored when the light supply exceeds that required to achieve the maximum relative growth rate for the nutrient limited growth rate, RGR_{max}. Konopka and Schnur (1980) obtained carbohydrate to protein ratios four to seven times higher in cultures limited by nitrogen, phosphorus or sulphur, than in non-limited cultures or those limited by carbon. In general, when major nutrients such as phosphorus or nitrogen limit cell growth, buoyancy decreases because carbohydrate accumulates, resulting in greater diurnal changes in buoyancy compared with nutrient replete cells (Chu et al. 2007). However associated turgor pressure increases can also collapse gas vesicles, especially if there is an accompanying rise in hydrostatic pressure due to sedimentation of cells to greater depths (Reynolds and Walsby 1975; Klemer 1978, 1991; Walsby 1987). The degree of gas vacuolation may also be reduced through molecular controls on gas vesicle production but this is species-specific. If nutrient limitation greatly depresses growth rate, then carbohydrate accumulation and buoyancy loss can occur even at low light intensities. Under severe and sustained nitrogen limitation reduced gas vesicle synthesis results in reduced buoyancy.

When all nutrients, including carbon, are present in abundance then buoyancy is largely a function of the irradiance intensity relative to the growth requirements of the cells. If the irradiance captured is less than that required to achieve maximum growth rate under the prevailing environmental conditions, then energy supply will be low relative to what could be utilised, carbohydrate reserves will be reduced, and cell buoyancy increased. In organisms like *Aphanizomenon flos-aquae*, where the degree of gas vacuolation is a function of the limiting energy supply, molecular processes will increase gas vesicle synthesis and enhance the positive buoyancy response (Utkilen et al. 1985; Konopka et al. 1987a; Kromkamp et al. 1988; Damerval et al. 1991). As discussed earlier for *Microcystis aeruginosa*, not all organisms show this molecular response to changes in illumination, suggesting species-specific.

When nutrient replete cells are exposed to irradiances close to saturating then they will grow at rates close to maximum and cellular elemental composition will approach the Redfield ratio (106C:16N:1P by atoms) (Hecky and Kilham 1988; Hecky et al. 1993). Under these conditions *Aphanizomenon flos-aquae* is generally negatively or neutrally buoyant (Konopka et al. 1987a; Kromkamp et al. 1988) whereas

Microcystis aeruginosa is positively buoyant (Kromkamp et al. 1988; Chu et al. 2007).

When nutrient sufficient cells are exposed to irradiance, carbohydrate stores increase with irradiance up to a maximum when photosynthesis is saturated. The enlarged carbohydrate store increases cell density, but molecular controls may decrease the rate of gas vesicle synthesis in some species. Gas vesicle collapse can occur from the resulting buildup of turgor pressure under light exposure, although this most often occurs synergistically with other buoyancy reducing mechanisms under normal light:dark cycles (Oliver and Walsby 1984; Kromkamp et al. 1986).

One of the first models to consider buoyancy was developed by Okada and Aiba (1983a, b). This model related density to rates of change of cell turgor pressure as follows:

$$\frac{dP}{dt} = \alpha(P_{max} - P)Q_{O_2} - \beta(P - P_{min})$$

where P is the turgor pressure of the cells (kN m^{-2}), P_{max} and P_{min} are the maximum and minimum assigned values of the turgor pressure, respectively, Q_{O2} is the photosynthetic activity of the cells (ml O_2 (g cell)$^{-1}$ h^{-1}), and α and β are proportionality constants for the respective rates of increase (g cell (ml O_2)$^{-1}$) or decrease (h^{-1}) in turgor pressure. Okada and Aiba (1983a) give values of P_{max} and P_{min} of 760 and 360 kN m^{-2}, respectively. While turgor pressure generated by osmotically active photosynthates or potassium ions is strong enough to collapse gas vesicles in some species (Allison and Walsby 1981; Oliver and Walsby 1984) cell density is altered most rapidly by the accumulation or loss of dense polysaccharides during photosynthesis and respiration (Kromkamp and Mur 1984; Utkilen et al. 1985; Kromkamp and Walsby 1990).

6.11.4 Sinking or Floating

The change in cell, colony or filament polysaccharide concentration alters cell density which then changes the rate of sinking or floating. In quiescent waters, i.e. under laminar flow conditions, the sinking or floating rate of phytoplankton can be calculated from the Stokes equation which describes sinking as the balance between the downward gravitational force from excess density and the buoyant viscous force provided by water:

$$v_s = \frac{2gr^2(\rho - \rho_w)}{9\eta\varphi}$$

where the terminal velocity of the organism (v_s) is dependent on gravitational acceleration (g), the size of the organism estimated as the radius (r) of a sphere of equal volume, the density of the organism (ρ), and the density (ρ_w) and viscosity (η) of the medium. A 'form factor' (ϕ) is included to adjust for the non-spherical shape of some organisms. This is defined as v_s/v_ϕ where v_ϕ is the terminal velocity of a sphere of equal volume and density to that of the organism. For some shapes the form factor is known from empirical relationships (McNown and Malaika 1950; Davey and Walsby 1985) but generally it has to be determined after measuring all other variables (Oliver et al. 1981). The Stokes equation is suitable for calculating sinking and floating velocities if the assumption of laminar flow is not violated, with particle-Reynolds number ($Re = 2rv_s\rho/\eta$) not exceeding 0.5 (Walsby and Reynolds 1980; Reynolds 1987). As a consequence the equation will not be reliable for large phytoplankton colonies where $r > 300$ µm (Reynolds 1987).

The Stokes function indicates that velocity is related to the square of the particle radius and is greatly enhanced by increased size. The direction of movement, as well as the velocity, is a function of the density difference between the particle (cells, trichomes or colonies) and the surrounding medium, while the shape of the particle may enhance or retard its motion. The large size range of the cyanobacteria, coupled with their ability to alter density, results in a wide range of floating and sinking velocities. In contrast, most freshwater eukaryotic micro-algae have a cell density greater than that of water and only the motile, flagellate species have any means of negating their propensity to sink. A notable exception to this is the green alga *Botryococcus* that can become buoyant after producing and accumulating oils.

Kromkamp and Walsby (1990) conceived a model to explain buoyancy regulation in a culture of *Oscillatoria* which had previously been incubated at an irradiance flux E of 10 µmol m^{-2} s^{-1}:

$$\frac{d\rho}{dt} = c_1 \frac{E}{K_E + E} - c_3$$

where $d\rho/dt$ is the rate of density change (kg m^{-3} min^{-1}), c_1 is the rate coefficient for rate of increase in density with time (given as 0.132 kg m^{-3} min^{-1}), c_3 is the minimum rate of decrease in density with time (given as 0.023 kg m^{-3} min^{-1}) and K_E is the half saturation constant for the density response to irradiance (kg m^{-3}). The Michaelis-Menten form of this equation produces a hyperbolic response of rate of change of density with increasing irradiance up to a maximum assigned rate of density increase ($c_1 = 0.109$ kg m^{-3} min^{-1}). When transferred to the dark the change in density of the culture can be described by:

$$\frac{d\rho}{dt} = c_2 E_a - c_3$$

where E_a is the previous irradiance (μmol m^{-2} s^{-1}) and c_2 is the light dependent rate coefficient for density change.

Howard et al. (1996) and Visser et al. (1997) included intracellular carbon dynamics to connect irradiance to carbohydrate accumulation and density fluctuations. Visser et al. (1997) hypothesised that the Kromkamp and Walsby (1990) model did not adequately describe density change at high light intensities because photoinhibition was not considered. Further experimentation by Visser et al. (1997) involving exposure of *Microcystis* to light intensities up to 1,500 μmol m^{-2} s^{-1} revealed photoinhibition of carbohydrate accumulation at $E > 1{,}374$ μmol m^{-2} s^{-1}, described by:

$$\frac{\mathrm{d}\rho}{\mathrm{d}t} = \left(\frac{N_0}{60}\right) I \mathrm{e}^{(-E/E_0)} - \ell$$

where N_0 is a 'normative factor' (0.0945 kg m^{-3} (μmol photon)$^{-1}$ m^2), E_0 is the value of E where $\rho(E)$ is maximal (μmol m^{-2} s^{-1}) and c_3 has been defined above but was assigned a value of 0.0165 kg m^{-3} min^{-1} to represent density change at $E = 0$ (Visser et al. 1997) compared with the value of c_3 of 0.023 kg m^{-3} min^{-1} used for *Oscillatoria* by Kromkamp and Walsby (1990). The above equation of Visser et al. (1997) was applied for values of photon irradiance above compensation levels (E_c, assigned as 10.9 μmol m^{-2} s^{-1}) while for $E < E_c$, the rate of density change was given as:

$$\frac{\mathrm{d}\rho}{\mathrm{d}t} = f_1 \rho_i + f_2$$

where ρ_i is the initial density (kg m^{-3}), f_1 regulates the rate of decrease in density (kg m^{-3} h^{-1}) and f_2 is a value corresponding to the rate of density change with no carbohydrate storage in the cells (kg m^{-3} h^{-1}). This equation takes into account that the rate of carbohydrate loss, and hence density decrease, is dependent upon the previous light history and the concentration of carbohydrate stored within cells (Stone and Ganf 1981). Beardall et al. (1994) observed that an increase in dark respiration following light exposure was dependent upon both the photon flux and the duration of light exposure; processes that are implicitly included in the model of Visser et al. (1997). Howard et al. (1996) used the change in density equations of Kromkamp and Walsby (1990) coupled with a simple hydrodynamic model as the basis for a model of cyanobacterial bloom formation known as SCUM. They considered the generation of ballast in response to "excess" carbohydrate accumulation (B) as:

$$B = P_{qi} - R - K$$

where P_{qi} is the photosynthetic rate for a given light intensity (mol C (mol C)$^{-1}$ s^{-1}), R is the respiration rate (assigned a value of 0.55×10^{-6} mol C (mol C)$^{-1}$ s^{-1} according to Reynolds (1990)) and K is growth (assigned a maximum value of 5.5×10^{-6} mol C (mol C)$^{-1}$ s^{-1} from Reynolds (1990)). Once B is assigned it is possible to determine the change in cell density resulting from the addition of carbohydrate as ballast:

$$\frac{\mathrm{d}\rho_{cell}}{\mathrm{d}t} = \frac{(B_g C_{cell})}{67}$$

where ρ_{cell} is cell density (g C μm^{-3} s^{-1}), B_g is $2.38B$ (mol C (mol C)$^{-1}$), C_{cell} is the carbon content per cell (14×10^{-6} g C) and 67 (μm^3) represents an assigned cell volume for *Microcystis*. If there is not sufficient photosynthetic carbohydrate generated then ballast is not accrued to increase density, and the carbohydrate is used to support growth and offset losses from respiration.

The increased rate of respiration following high irradiance can lead to cells with different rates of density change and productivity at a given irradiance, depending upon prior light history (Beardall et al. 1994; Ferris and Christian 1991). Patterson et al. (1994) used a particle tracking model to attempt to include previous light history in a model of cyanobacterial growth but they did not explicitly include a dynamic component of buoyancy regulation in their model.

Wallace and Hamilton (1999) extended the previous buoyancy models of Kromkamp and Walsby (1990), Howard et al. (1996), Visser et al. (1997) by including two additional terms; a transient response term τ_r and a dependence on the previous rate of density change ($\mathrm{d}\rho(0)/\mathrm{d}t$). The induced density changes represent a physiological adjustment time for cells to respond to variations in irradiance, observed to be c. 20 min ($\tau_r = 20$ min). Their equation for change in density with time for the case where $D(0) = 0$ (i.e., starting conditions in which density is constant) took the form:

$$\frac{\mathrm{d}\rho}{\mathrm{d}t} = \left(c_1 \frac{E}{K_E + E} - c_3\right)\left(1 - \mathrm{e}^{-(\mathrm{t}/\tau_r)}\right)$$

Their model was calibrated with measured data to give $c_1 = 0.0427$ kg m^{-3} min^{-1}, $c_3 = 4.6 \times 10^{-6}$ kg m^{-3} min^{-1} and $K_E = 530$ μmol m^{-2} s^{-1}.

One of the major difficulties with the idealised representations of buoyancy regulation presented above is the ability to include a light history term for cells as they are advected and diffused between the layers or compartments that are typically used in hydrodynamic models. To begin with, a model is required to describe the irradiance at a given water depth:

$$E(z) = E(0)\mathrm{e}^{-\varepsilon z}$$

Where $E(0)$ is the surface irradiance corrected for albedo, z is water depth (positive downward) and ε is the vertical attenuation coefficient for broadband photosynthetically available radiation (400–700 nm). Various formulae

(e.g., Luo et al. 2010) are commonly used to distribute both total daily irradiance and albedo in a sinusoidal pattern as a function of time of day, as well as to construct irradiance from measurements based on, for example, global horizontal radiation, cloud cover and an assigned fraction of photosynthetically active radiation (0.46; Kirk 1991).

The vertical attenuation coefficient, ε, is affected by five constituents of natural water, that is water itself, dissolved organics, phytoplankton, detritus and inorganic solids. The way in which cyanobacteria affect ε can be described by:

$$\varepsilon = \varepsilon_O + K_C C$$

where ε_O is the vertical attenuation contributed by water and other natural constituents including other phytoplankton, C is a variable describing biomass of cyanobacteria and K_C is a specific attenuation coefficient normalised for the cyanobacterial biomass unit. The literature on specific light attenuation coefficient values for cyanobacteria and other phytoplankton is characterised by both a wide range of units and values.

Litchman (2003) gives species-specific light attenuation coefficients derived in the laboratory of 5.2×10^{-6} cm^2 filament^{-1} for *Phormidium luridum* and 1.7×10^{-6} cm^2 filament^{-1} for *Anabaena flos-aquae*. Different units have been used in modelling studies; 3.4 mm^2 cell^{-1} for *Microcystis* (Jöhnk et al. 2008) and 10 m^2 g^{-1} phytoplankton phosphorus for filamentous cyanobacteria (Scheffer et al. 1997). Values of K_c vary not only between species but also within species. Ganf et al. (1989) found that values of K_C when *Microcystis aeruginosa* was the dominant species in an artificial lake varied at 0.2 m depth from 0.0138 to 0.0106 m^2 mg^{-1} chlorophyll *a* for individual cells and colonies, respectively. At greater depths they found K_C values of 0.14 and 0.11 m^2 mg^{-1} for cells and colonies, and 0.029 and 0.020 m^2 mg^{-1} for non-vacuolate cells and colonies, respectively. They attributed gas vacuoles to be responsible for around 80% of the light scattering arising from their observed *Microcystis* cells and colonies.

6.11.5 Colony Formation and the Effect on Buoyancy

Sinking/floating velocity increases with the square of the radius, according to Stokes Law, and so colony size plays an important role in determining the vertical position of colonies and filaments in the water column. While carbohydrate accumulation and loss occur in response to light this may not be expressed as large diurnal migrations in small colonies that barely move in the water column (Visser et al. 1997). As colony size increases, the amplitude of migration increases and in a quiescent water body colonies may oscillate between the surface and depth several times within a single day (Kromkamp and Walsby 1990; Visser et al. 1997). Slow vertical migration has several implications for small colonies. Cells stranded near the surface can experience photoinhibition which decreases the ability for vertical migration as carbohydrate ballast accumulation is impaired (Ibelings and Maberly 1998; Brookes et al. 2000; Wallace and Hamilton 1999). Cells at depth may experience extended light limitation. In both cases there is an inability to overcome the vertically separated resources of light and nutrients (Ganf and Oliver 1982).

Typical vertical migration velocities for cyanobacteria range from 7 μm s^{-1} for *Planktothrix rubescens* up to 3,000 μm s^{-1} for *Microcystis aeruginosa* (Reynolds et al. 1987), influenced strongly by the size of filaments or colonies. In filamentous forms, the length of filaments can increase with growth and the production of new cells within the trichome. This mechanism of increasing filament size is limited by the rate of cell division and is relatively slow. The aggregation of filaments to produce larger units offers the potential to rapidly change the size of the unit and significantly increase the floating velocity. Filaments of *Anabaena circinalis* have been observed to aggregate at low light intensities (Brookes et al. 1999). The greatest axial linear dimension increased in the dark from 90.3 to 202.9 μm which effectively increased the floating velocity from 0.39 to 202.9 m h^{-1} (108–56,360 μm s^{-1}).

The aggregation of cells into colonies may result from modification of charge or hydrophocity and increases in colony size are generally associated with increased exudation of polysaccharides (Yang et al. 2008). Studies have demonstrated that microcystin and lectins (Kehr et al. 2006) or the microcystin-related protein MrpC (Zilliges et al. 2008) may play a role in inter-cellular aggregation in *Microcystis*. Not only does colony formation enable large vertical migrations, but it may also be a mechanism by which to avoid grazing losses (Sect. 6.12).

6.11.6 Within-Population Variability

In populations where buoyancy loss is observed often a significant proportion of the population retains buoyancy as some cells have sufficient gas vesicles to counteract the accumulation of dense polysaccharide. Persistently buoyant *Anabaena lemmermannii* and *Anabaena minutissima* filaments were observed in Lake Windermere (Walsby et al. 1991) and Lake Rotongaio, respectively (Walsby et al. 1987, 1989). It is interesting to note that the majority (88.9%) of *Anabaena minutissima* suspended in bottles at the water surface retained buoyancy following 9 h of daylight, while at the end of the light period on the second day of incubation 44.9% of filaments still remained buoyant (Walsby et al. 1991).

Persistent buoyancy is often associated with nutrient enrichment. Brookes et al. (1999) found that nutrient enriched *Anabaena circinalis* displayed significantly attenuated buoyancy loss relative to a treatment with no nutrients added. Similarly, *Aphanizomenon* with nutrients added maintained buoyancy after a 5 h light incubation (Klemer et al. 1995). Brookes et al. (2000) used flow cytometry to investigate gas vesicle volume and metabolic activity of individual cells of *Microcystis aeruginosa*. Even in culture populations, which are generally considered to be homogeneous, there was considerable variability of both gas vesicle volume and photosynthetic rate, presumably a function of cell age and stage of cell division.

The variation in buoyancy response highlights the considerable physiological variability in individuals in natural populations. Accounting for this variability in models is challenging because the attributes of individual colonies need to be predicted through time. This is possible with particle tracking models. Attributes such as photosynthesis, buoyancy regulation, nitrogen-fixation and growth can be predicted for the individual cells or colonies using this method, not only for an 'average population'. Furthermore these models enable the modelling of vertical particle trajectories in turbulent aquatic environments where the turbulent mixing is non-uniform (Ross and Sharples 2004). Beckman and Hense (2004) have developed a model of vertical migration that is dependent upon internal nutrient stores which when linked to a particle tracking model could predict the buoyancy response of individuals based on the cellular nutrient status. Advances with this modelling strategy would satisfy the need to move toward a more physiologically based approach rather than assigning rate constants for populations irrespective of cellular nutrient status.

Buoyancy may also play a role in recruitment and loss of colonies from sediments. Temperature controls on the respiration rate can lead to more accumulation of carbohydrate in cooler water bodies. The accumulation of glycogen results from a lower rate of protein synthesis at low temperatures and has been implicated in the loss of colonies from the water column in autumn as water temperatures decrease (Visser et al. 1994). The recruitment of cells from sediments may also be facilitated by buoyancy although Verspagen et al. (2004) suggest that recruitment is more likely to result from wind-driven resuspension.

6.11.7 Effects of Turbulence

Buoyancy maintains cyanobacterial cells in suspension while its regulation enables them to move vertically through the water in response to changing growth conditions. However the extent to which gas-vacuolate cyanobacteria can control their vertical distribution is also a function of the turbulent mixing regime. Steinberg and Hartmann (1988) analysed cyanobacterial distributions across a number of waterbodies and concluded that turbulence in lakes and rivers should be regarded as a special quasi-resource that can be differentially exploited by various phytoplankton in a manner analogous to nutrients or light (Reynolds and Walsby 1975; Harris 1986). Consequently, research into hydrodynamics has significantly advanced our understanding of the effects of turbulent mixing on the growth and distribution of phytoplankton, and particularly in selecting for gas-vacuolate cyanobacteria.

6.12 Population Losses

Factors promoting algal blooms are relatively well documented, but bloom collapse is often less clear. For example, Thompson et al. (2003) were able to document the bloom formation of *Anabaena* spp. in the Canning River, Western Australia, but were unable to ascertain the reasons behind the collapse of the bloom except to say that low dissolved inorganic phosphorus, low inorganic carbon, low light and high oxygen concentrations were probable causes. Such outcomes are not uncommon where investigations focus on the abiotic factors that promote blooms in anticipation that reversal of these factors will cause the demise of a bloom. Grazing by zooplankton and protozoans impact upon phytoplankton populations (Canter and Lund 1968; Oliver and Ganf 2000; Sommer et al. 2003; Reichwaldt and Stibor 2005) whilst the impact of infection by cyanophages, whether by bacteria, bacteriophages, fungi, or viruses can also be significant. As noted also in Chap. 7, copepods, cladoerans and rotifers can graze phytoplankton, but many cyanobacteria have specific features that make them less palatable to grazing than other phytoplankton. Large filamentous or colonial cyanobacteria may mechanically interfere with grazers as a result of their size or mucilage, while toxins produced by some cyanobacteria have also been implicated in having an allelopathic function against grazers (Chap. 7). Yang et al. (2006) propose that colony formation in *M. aeruginosa* is a defense strategy against grazing flagellates due to a mismatch in size between the colonies and the grazers and may be considered as an inducible defense against flagellate grazing. It appears that while toxins may be effective against metazoan zooplankton (Yang et al. 2006; Liu et al. 2005; Ghadouani et al. 2003) colony size may also play a role. The palatability and capacity for grazers to exert significant controls of very dense, potentially toxic blooms in which cells are mostly aggregated into large colonies, such as *Microcystis* blooms in Lake Taihu, China (Fig. 6.11), is questionable. There is, however, some indication that *Microcystis* cells can also respond to grazers by aggregating into colonies in the presence of purified microcystin toxin (Sedmak and Elersek 2006), and in cultures exposed to spent *Daphnia* media and disrupted *Microcystis* cells (Becker 2010).

Fig. 6.11 Bloom of *Microcystis* spp. in Lake Taihu, Jiangsu Province, China, in October 2007. This followed a massive lake-wide spring-summer (May–July) bloom, which severely interfered with the water supply for several million residents in nearby Wuxi City. Lake Taihu is well known for such *Microcystis* blooms at this time of year

Shilo (1970, 1971) drew attention to biological agents that cause cell lysis in cyanobacteria and Daft and Stewart (1971) suggested that over 40 cyanobacterial strains, including bloom forming species, were susceptible to bacterial isolates that cause cell lysis. A combination of abiotic factors and pathogens may elicit cellular responses such as differentiation, cell death, cell-cycle arrest, formation of resting stages and asymmetric cell division leading to death of the older cell (Franklin et al. 2006; Chap. 21).

Several researchers (e.g. Mann 2006; Raven 2006; Middelboe et al. 2008) have recognised recently that bloom collapse may be due to biotic as well as abiotic factors or a combination of both. Biotic factors include fungal infection, viruses, bacteriophages, programmed cell death and glycogen accumulation that decrease rates of photosynthesis but increase respiration. There are a myriad of terms to describe cell death but Franklin et al. (2006) provide a useful set of definitions: natural cell death, programmed cell death, apoptosis, lysis, necrosis, paraptosis and senescence.

6.12.1 Chytrid Infections

Chytrids, mainly aquatic fungi, belong to the phylum Chytridiomycota, class Chytridiomycetes, order Chytridiales. Two groups of zoosporic fungi once included among chytrids are now classified as separate phyla, Blastocladiomycota and Neocallimastigomycota. The importance of these fungi is illustrated by the discovery of the frog chytrid (*Batrachochytrium dendrobatidi*) that has caused the widespread death of amphibians in Australia (Berger et al. 1998). Chytrids typically have a haploid (n) and a diploid (2n) phase. It is the flagellated diploid zoospore that attacks algal cells. Kagami et al. (2007) have reviewed fungal parasitism on phytoplankton and provide some interesting insights into identification, visualisation and culturing of chytrids, as well as information on the likelihood of infection, host specificity and fungal epidemics. They describe how fungal parasitism may influence food chains and introduce a new pathway termed the 'Mycoloop'. This explains how large and often inedible phytoplankton are infected by parasitic fungi (particularly Chytrids) whose zoospores consume nutrients from their phytoplankton hosts which are subsequently eaten by zooplankton, thus extending our knowledge of both algal death and nutrient cycling. They conclude that estimates of zoospore abundance and the prevalence to infection of phytoplankton are urgently needed if the collapse of algal blooms is to be understood, and advocate the adoption of the model parameters described by Brunning et al. (1992).

Anabaena flos-aquae is a common bloom-forming cyanobacteria of freshwaters and is susceptible to infection by a chytrid. Sigee et al. (2007) used a simple dye (Evans Blue) to ascertain whether cells present in a eutrophic lake (Rostherne Mere, UK) were alive, senescent or dead. Vegetative cells and akinetes were susceptible to infection with filamentous colonies disintegrating and 30% of the akinetes died. In the same paper the authors investigated the death of *Microcystis aeruginosa* and came to the conclusion that death was primarily a response to adverse conditions with no evidence of fungal infection, but moribund cells underwent programmed cell death.

Although chytrids may infect various algae their presence does not always lead to significant decreases in cell numbers. Takano et al. (2008) showed that in Lake Shumarinai, Japan, two chytrid types infected *Anabaena smithii,* one on akinetes and the other on heterocysts. Although maximum parasitism of filaments was 20.6%, the abundance of *A. smithii* did not decrease. They suggested that nitrogen fixation could be affected but the concentration of available nitrogen was too high to detect any effect.

6.12.2 Programmed Cell Death

Marine cyanobacteria of the genera *Trichodesmium* often form extensive blooms that may disappear abruptly within 1–2 days as cell lysis and decomposition occur. Ohki (1999) has suggested that this is due to bacteriophage infection. However, Berman-Frank et al. (2004) have implicated autocatalytic cell death triggered by nutrient stress (Segovia et al. 2003) as a likely mechanism. Autocatalytic cell death is analogous to programmed cell death (PCD) in multi-cellular organisms and refers to an active, genetically controlled, cellular self-destruction mechanism driven by a series of biochemical events. They showed that in cultures consisting

primarily of *Trichodesmium erythraeum* programmed cell death was initiated by a combination of Fe and P limitation at high irradiances.

Franklin et al. (2006) suggest that freshwater environments are ideal habitats for the investigation of PCD because phytoplankton are relatively abundant and blooms are frequent and more accessible than in the marine environment. They found that the death of *Anabaena flos-aquae* appeared to be related to oxidative stress and nutrient limitation leading to PCD. It appears as though PCD is correlated with oxidative stress; an imbalance between the synthesis of reactive oxygen and the cell's ability to maintain a normal internal redox potential. This can be caused by nutrient deficiency, viral infection or other stressors. For example in Saint Lucie River Estuary, Florida, a population of *Microcystis aeruginosa* became stressed due to an increase in salinity with detectable toxin levels rising by 90%. This was accompanied by a significant increase in the production of hydrogen peroxide compared with unstressed cells, with levels similar to those that have induced PCD elsewhere (Ross et al. 2006). Franklin et al. (2006) suggest that free radicals such as hydrogen peroxide may act as internal signals promoting PCD.

6.12.3 Cyanophages

Viruses are very abundant in freshwater and can reach concentrations of 10^6–10^9 cells mL^{-1}. They are known to cause the death of both eukaryotic and prokaryotic, marine and freshwater phytoplankton as well as macroalgae (Brussaard 2004; Chap. 21). They were thought to be host specific, but Deng and Hayes (2008) have shown that cyanophages isolated from Lake Zurich, Switzerland and Cotswold Water Park, U.K., had a very broad host range and were able to infect *Anabaena, Microcystis* and *Planktothrix*. Viruses can control cyanobacterial populations. Tucker and Pollard (2005) isolated *Microcystis aeruginosa* from Lake Baroon, Queensland, Australia. The growth rate of *M. aeruginosa* under optimal conditions of light and nutrients was 0.023 h^{-1} (0.552 day^{-1}) and reached a density of 1.09×10^7 cells mL^{-1} after 6 days. When *M. aeruginosa* was incubated with a series of tenfold dilutions of the natural viral community the final concentration of *M. aeruginosa* cells fell. At the natural lake viral concentration the final biomass of cells was reduced by 95% and this was correlated ($r^2 = 0.95$) with the final number of viral like particles (10^7 mL^{-1}) present.

However, not all viral infections produce the anticipated result. Cell numbers of *Cylindrospermopsis raciborskii* from Lake Samsonvale in southeast Queensland, Australia, incubated with natural viral like particles (VLP) decreased by 85% after 5 days and this was correlated with a step-wise increase in VLP (Pollard and Young 2010). However, as the cells lysed the filaments of cells split into smaller units which still had viable cells. They suggest that this process may aid in the re-distribution of *C. raciborskii* throughout the lake. Further, phytoplankton may have a defence mechanism against parasitic viruses that is dependent upon the release of dissolved organic matter which attracts flagellates that eat the parasitic viruses (Murray 1995).

Viral ecology is an expanding science (Chap. 21) and viruses can be a major agent for the death of host phytoplankton. However, whether or not viral ecology can be used to manage cyanobacterial blooms is still problematic because of the rapid selection of resistant host strains (Wilhelm and Matteson 2008) and the only examples of artificial control of cyanobacteria by phages is at an experimental system scale (Chap. 21). Similarly, models of effects of cyanophages are still in their infancy, but have yielded useful hypotheses about the dependence of viral impacts on the physiological status of cells (Gons et al. 2006; Chap. 21).

6.13 Water Movement and Blooms

It is possible to separate three main categories of models that characterise the effects of water movement on cyanobacteria populations. One category includes the application of hydrodynamic models to explicitly quantify the effects of advective transport and turbulent mixing processes on cyanobacteria populations. The resulting models are considered in more detail in Sect. 6.14. Another category uses various numerical indices to denote, in combination with phytoplankton physiological attributes, when water stability may be conducive to formation of blooms. The last uses conceptual models as simple as asking are hydrodynamic/light conditions 'correct' for a noxious cyanobacteria bloom (Elser 1999). This type of conceptual model has been used as part of a decision support tree approach to help step through a set of prequisite environmental conditions that might promote a bloom (Elser 1999; Oliver and Ganf 2000). Exploration of the 'correctness' of the hydrodynamic environment and light climate for blooms leads naturally to the approach used in category two of seeking numerical indicators.

A process based model of bloom potential of cyanobacteria is given by Humphries and Lyne (1988). Their model was the first to consider positive buoyancy (i.e. rising particles) in a growth-diffusion model application to the vertical dimension. The model examines the interplay of turbulence in the surface mixed layer and positively buoyant cells or colonies of *Microcystis.* At short time scales buoyancy plays a far more important role in determining the vertical concentration profile. If turbulent mixing is intense then populations of both positively-buoyant and negatively-buoyant phytoplankton will be homogenised through the surface mixed layer. If turbulent mixing is weak, then populations can become disentrained from the predominant turbulent eddies and

strong concentration discontinuities ensue. Humphries and Lyne (1988) used a non-dimensional time scale for diffusion in the mixed layer relative to the sinking or floating time scales of populations. This diffusion or mixing time scale T_{mix} was given as:

$$T_{mix} = \frac{z_m^2}{K}$$

where z_m is the depth of the surface mixed layer (m) and K is the vertical coefficient of eddy diffusivity ($m^2\ s^{-1}$). The characteristic time scale T_v (s) for cells sinking or rising over the water depth is:

$$T_v = \frac{z_m}{\nu_s}$$

where ν_s is the sinking or rising velocity ($m\ s^{-1}$). The variable *Pe*, known as the Péclet number, gives a value for the rate of diffusion relative to sinking or rising:

$$Pe = \frac{T_{mix}}{T_v}$$

A value of $Pe > 1$ defines when the influence of sinking or floating begins to dominate the vertical distribution of cells, as opposed to the homogenising effect of turbulent diffusion. Huisman and Hulot (2005) suggested that at $Pe > 10$ *Microcystis* will completely disentrain from the turbulence and can then form surface blooms. Values of the Péclet number also have relevance to artificial mixers or destratification for control of cyanobacterial blooms (see Reynolds et al. (1984) and Visser et al. (1996), for applications of artificial mixing for this purpose). Increasing the rates of vertical mixing through artificial mixing or destratification effectively reduce T_{mix} and therefore *Pe*, with the outcome that *Pe* will remain sufficiently low so as to prevent cells or colonies from accumulating at the surface.

Another approach to assessing the effect on cyanobacteria distributions of water movement in the surface mixed layer is used by Howard et al. (1996) in the "SCUM" model. This model uses the Wedderburn number (W) which reflects the balance between surface wind stress and the energy inherent in the water density gradient (Imberger and Hamblin 1982), to attempt to capture the temporal dynamics of the depth of the surface mixed layer (h) using the equation:

$$h^2 = \frac{W \rho_w v_* L}{g \Delta\rho}$$

where ρ_w is a standard reference density for water, $\Delta\rho$ is the density gradient that separates water of uniform density at the water surface from the water density at the base of the gradient, L is the fetch length (the length of the lake or reservoir at the density gradient in the direction of the wind), g is the gravitational acceleration constant and v_* is the average turbulent water velocity given by:

$$v_*^2 = \frac{C_d \rho_a U_{10}^2}{\rho_w}$$

where C_d is the drag coefficient (1.3×10^{-3}), ρ_a is the density of air ($1.2\ kg\ m^{-3}$) and U_{10} is the wind velocity at 10 m elevation ($m\ s^{-1}$). When the calculated value of W falls below 1, the mixed layer depth is recalculated with $W = 1$ (i.e. wind stress and buoyancy forces are approximately equal). Within this mixed layer the cyanobacteria colonies are redistributed with a random-walk model to reflect the dominant influence of turbulent mixing over the rising velocities of colonies within this layer. A random walk model for cyanobacteria motion in the surface mixed layer has been used in a similar way by Patterson et al. (1994) and Wallace and Hamilton (2000) but for those cases the transport and mixing of water was accomplished with the hydrodynamic model DYRESM (Imberger and Patterson 1981). Wallace and Hamilton (1999) examined other theoretical cases of cyanobacteria movement within the surface mixed layer represented by coherent paths of Langmuir circulation, using a model by Buranathanitt et al. (1982), as well as a case with no water movement at all. Each of these cases demonstrated the critical role of colony size where larger colonies were more easily able to become disentrained from the turbulent mixing to form surface blooms. The entrainment of cyanobacteria can also be related more directly to wind speed (Webster and Hutchinson 1994), with values above a critical value of 2–3 $m\ s^{-1}$ defining when colonies are entrained into the turbulent surface layer rather than floating to the surface. Otherwise, colonies can float within a surface film and marked horizontal gradients ensue that can lead to blooms on leeward shores under continuous directional forcing by wind (Figs. 6.1c and 6.1h). This exponential accumulation of colonies downwind has a length scale that is inversely proportional to the colony flotation speed (Hutchinson and Webster 1994).

Turbulent mixing itself can also play a role in colony size, as shown in grid-stirred tank experiments with *Microcystis aeruginosa* by O'Brien et al. (2004). They obtained a significant relationship ($r^2 = 0.85$, $p < 0.01$) between turbulent dissipation and colony size corresponding to occurrence of smaller colonies with increasing turbulence. The variability of, and interactions amongst wind speed, water turbulence, and cyanobacteria buoyancy (Sect. 6.11) and colony size, make it highly challenging to adapt process based models for prediction of blooms and hence the application of simpler indicators of bloom potential discussed above.

6.14 Ecosystem Models That Include Water Movement

In a review of lake ecosystem models Mooji et al. (2010) indicate the large number and wide diversity of approaches that have been used to model phytoplankton biomass at the ecosystem scale. A smaller subset of these models has been used to model explicitly the biomass or chlorophyll *a* associated with particular species of cyanobacteria or of the phylum collectively. Many of these models have an underpinning hydrodynamic model to account for the effects of advection and diffusion in transporting cyanobacteria. The most complex of these models is three-dimensional (3-D) and generally represents a lake ecosystem by Cartesian coordinates (x, y, z) in an orthogonal coordinate system where the coordinates are perpendicular to each other. In a curvilinear coordinate system the orthogonal coordinate system is modified by transforming lines to curves. Several applications of 3-D hydrodynamic models (e.g. ELCOM) have used mathematical formulae to transform between orthogonal and curvilinear coordinate systems (Hodges and Imberger 2001; Venkatesh et al. 2005). A non-Cartesian coordinate system allows for flexibility of coordinate positions so that the model can be adapted to better resolve specific areas within a model domain and reduce distortion of the physical domain, but with considerable increase in mathematical complexity. Dispersion of a pollutant or flow in spatially constricted areas such as channels provide examples where non-Cartesian coordinates can better capture the dynamics than a Cartesian coordinate system for a given computational effort. In the case of cyanobacteria it would be expected that these models may be more suitable for capturing fine-scale shoreline accumulations often characterising blooms in lentic waters.

Most 3D models have a set of governing equations for transport of mass and momentum, and surface thermodynamics. Commonly the transport terms are based on numerical solutions of the unsteady Reynolds-averaged Navier-Stokes (RANS) equations which describe the velocity of a fluid in 3D space. The numerical approximation in the RANS equations commonly requires closure via a turbulence model in order to include the turbulent properties of the flow. With increasing computational power applications of these models for hindcasting or predicting cyanobacterial blooms are becoming more routine (Robson and Hamilton 2004) and circumvent some of the issues associated with models of reduced dimension which have used vertical or horizontal averaging and will not capture horizontal wind transport of colonies (Burger et al. 2007) or surface blooms associated with short-term stratification events (MacIntyre 1993), respectively. Nevertheless these models of reduced dimension have yielded important insights into bloom dynamics including applications with the 1D (horizontally averaged) DYRESM model to understand interactions amongst water movement and cyanobacteria colony size and density change (Wallace and Hamilton 1999, 2000; Wallace et al. 2000) and a 1D model to examine competition amongst cyanobacteria and negatively buoyant phytoplankton (Huisman et al. 2004).

It is impossible to do justice to the many coupled hydrodynamic-ecological models that could potentially be used for the purpose of modelling cyanobacteria. A few of the better known models are discussed briefly below, with limited examples of applications for cyanobacteria populations. ELCOM-CAEDYM is a 3D model that has been used to simulate a major bloom of *Microcystis aeruginosa* in the Swan River estuary in Western Australia (Robson and Hamilton 2004). The model was used to capture a transition following a major rainfall event of brackish water displacement by freshwater and the resulting bloom. The seven-compartment phytoplankton model used in CAEDYM allows for specific species or groups of cyanobacteria to be simulated concurrently with other phytoplankton groups, to capture seasonal patterns of succession (Bruce et al. 2006; Burger et al. 2007) or changes that may be expected with a warming climate (Trolle et al. 2010). Figure 6.12 shows an application of the ELCOM-CAEDYM model to Lake Rotorua, New Zealand, with daily simulation output of surface concentrations of cyanobacteria (represented as equivalent chlorophyll *a* concentration) changing rapidly. These rapid changes are induced mostly by wind acting on the surface mixed layer where buoyant populations of cyanobacteria accumulate. Some evidence of a mid-lake concentration is also sometimes observed within this lake (Fig. 6.13). In this figure cyanobacteria (*Microcystis* sp.) were numerically dominant in the phytoplankton population and chlorophyll fluorescence readings across the lake (vertically and horizontally) were interpreted as representative of the relative biomass of cyanobacteria. PROTECH (Phytoplankton RespOnses To Environmental CHange) is a phytoplankton model that has most commonly been used with a one-dimensional model of mixing and transport to examine the seasonal succession of different phytoplankton groups in lakes (Reynolds et al. 2001). Simulations have demonstrated the likelihood of increasing relative abundance of cyanobacteria in a warmer climate (Elliot et al. 2005, 2006), which has subsequently been reinforced in modelling by Trolle et al. (2010). PROTECH can simulate up to 10 phytoplankton species or groups but has the most extensive phytoplankton parameter library that includes over 100 phytoplankton species. Many of these parameters make use of a morphological trait of surface area to volume of individual cells or colonies in order to assign key physiological characteristics such as growth and respiration rates to individual species (Kruk et al. 2010). Delft3D-ECO is a 3-D hydrodynamic-ecological model that can incorporate an additional model known as BLOOM (Los et al.

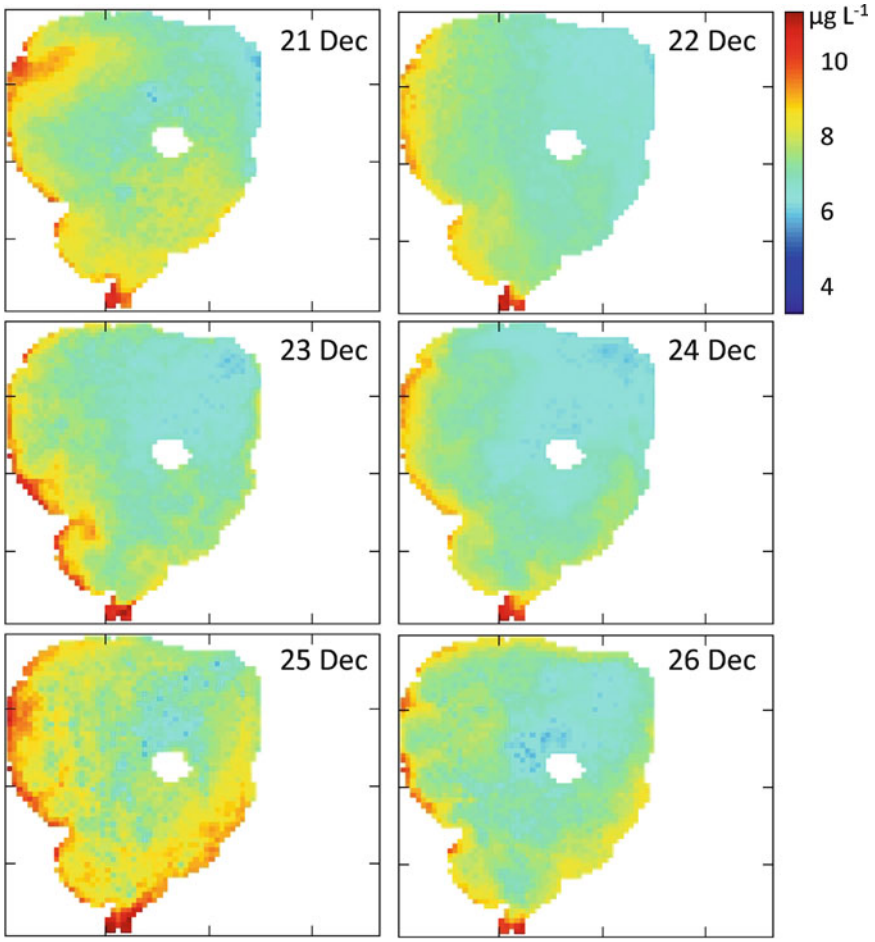


Fig. 6.12 Daily output (midday, 21–26 December 2007) of cyanobacteria (as chlorophyll *a* concentration) derived from a 3-dimensional hydrodynamic-ecological model (DYRESM-CAEDYM) of Lake Rotorua, North Island, New Zealand. A colour scale is on the right and represents cyanobacteria as equivalent chlorophyll *a* concentration. The lake has an area of 80 km^2

2008; Los 2009) which explicitly includes state variables of *Microcystis, Aphanizomenon* and *Planktothrix*. SALMO (Simulation of an Analytical Lake Model)-HR is a 1D (horizontally averaged) model that includes at least three functional phytoplankton groups (Recknagel et al. 2008). There are many models of various levels of complexity and resolution which have different levels of parameterisation either inherent in the model (e.g. PROTECH) or provided by a user as part of the calibration process based on assigned ranges. Many of the current models seek some balance between lumping phytoplankton into one or a few biomass categories and seeking to capture dynamics of species that are of intense interest; for example particular species of cyanobacteria that may form blooms and be toxic. As noted by Harris (1994), however, these two models can be of very different types, pointing to the difficulty of generalising phytoplankton populations as a group for ease of parameterisation versus attempting mechanistic descriptions to capture the characteristics of the many different species and their interactions found in an ecosystem. Thus capturing the seasonal succession of cyanobacteria through a growing season still remains a challenge for the foreseeable future for aquatic ecosystem models attempting quantitative predictions of cyanobacterial blooms. Furthermore, life cycle strategies of overwintering and akinete formation and germination are mostly poorly captured and yet are critical to the survival strategy of many cyanobacteria (Chap. 7). Quantitative modelling of the environmental triggers for benthic life cycle stages is largely unexplored and should be addressed for a more complete understanding of the triggers leading to cyanobacterial blooms.

6.15 Conclusions

Cyanobacteria have a number of key attributes that enable them to be able to accumulate in large concentrations and form surface blooms under certain conditions. A coalescence of favourable conditions for bloom formation includes, but is not limited to: adequate nutrients to support cell replication including nitrogen fixation in a subset of cyanobacteria when cellular nitrogen demands are not met; buoyancy imparted by gas vacuoles; alterations in photosynthetically-fixed carbohydrate that acts as ballast; aggregations of cells as colonies

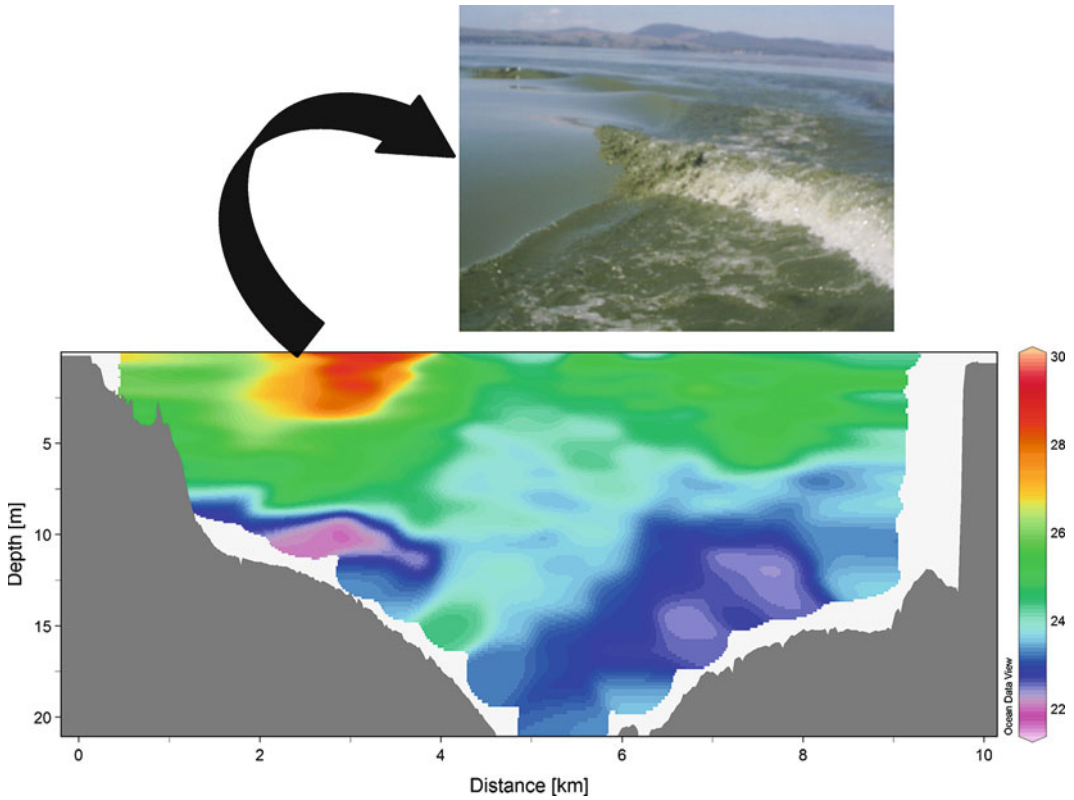


Fig. 6.13 Cross-sectional profile (depth 21 m, distance across lake c. 10 km) of chlorophyll fluorescence in Lake Rotorua, North Island, New Zealand on 10 March 2004 (lower). *Anabaena* sp. was strongly dominant amongst the phytoplankton assemblage and the colour scale is an indicative concentration in µg chlorophyll *a* L^{-1}. Note the high concentration (bloom) at a horizontal location of 2.5 km (The photo (upper) was taken at this location (D. Burger, with permission))

or filaments to enhance buoyancy; and disentrainment from turbulent motions in the surface mixed layer of waterbodies. Furthermore the cyanobacteria are characterised by substantial capacity for photoacclimation and photoadaptation that enables them to maintain light harvesting efficiency and to avoid increased risk of photodamage associated with high surface irradiances that may occur in blooms. The complexity and diversity of these processes and their variability within and between species represents an enormous challenge for models attempting to forecast the occurrence of cyanobacterial blooms at the scale of whole waterbodies. At best these models can capture only a subset of the processes and species parameters necessary to predict successional sequences within and between cyanobacterial species and other phytoplankton. Nevertheless, as knowledge of cyanobacterial physiology grows, and with increases in computational power, models will extend beyond tools to test theoretical concepts and single response factors, and yield integrated, quantitative estimates of the timing, frequency and magnitude of cyanobacterial blooms. Models that incorporate the collapse of blooms when mediated by biotic factors (e.g. infection and or grazing) will be particularly useful. This degree of realism will allow these models to be used more routinely for quantitative predictions of cyanobacterial biomass with applications for strategies that could be used to influence the timing and magnitude of populations.

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References

Adams DG, Duggan PS (1999) Tansley review No. 107. Heterocyst and akinete differentiation in cyanobacteria. New Phytol 144:3–33

Agawin NSR, Rabouille S, Veldhuis MJW, Servatius L, Hol S, van Overzee HMJ, Huisman J (2007) Competition and facilitation between unicellular nitrogen-fixing cyanobacteria and non-nitrogen-fixing phytoplankton species. Limnol Oceanogr 52:2233–2248

Ahn C-Y, Joung S-H, Park C-S, Kim H-S, Yoon B-D, Oh H-M (2008) Comparison of sampling and analytical methods for monitoring of cyanobacteria-dominated surface waters. Hydrobiologia 596:413–421

Allen MM (1984) Cyanobacterial cell inclusions. Annu Rev Microbiol 38:1–25

Allison EM, Walsby AE (1981) The role of potassium in the control of turgor pressure in a gas-vacuolate blue-green alga. J Exp Bot 32:241–249

Alric J, Lavergne J, Rappaport F (2010) Redox and ATP control of photosynthetic cyclic electron flow in *Chlamydomonas reinhardtii* (I) aerobic conditions. Biochim Biophys Acta Bioenerg 1797:44–51

Andersen T (1997) Pelagic nutrient cycles. Herbivores as sources and sinks. Springer, Berlin/Heidelberg/New York, 280 pp

Arhonditsis GB, Qian SS, Stow CA, Lamon EC, Reckhow KH (2007) Eutrophication risk assessment using Bayesian calibration of process-based models: application to a mesotropic lake. Ecol Model 208:215–229

Aurora R, Hihara Y, Singh AK, Pakrasi HB (2007) A network of genes regulated by light in cyanobacteria. OMICS J Integr Biol 11(2): 166–185

Azevedo SMFO, Carmichael WW, Jochinsen EM, Rinehart KL, Lau S, Shaw G, Eaglesham GK (2002) Human intoxication by microcystins during renal dialysis treatment in Caruaru – Brazil. Toxicology 181–182:441–446

Bailey S, Grossman A (2008) Photoprotection in cyanobacteria: regulation of light harvesting. Photochem Photobiol 84(6):1410–1420

Baird ME, Emsley SM (1999) Towards a mechanistic model of plankton population dynamics. J Plankton Res 21:85–126

Baklouti M, Diaz F, Pinazo C, Faure V, Quéguiner B (2006) Investigation of mechanistic formulations depicting phytoplankton dynamics for models of marine pelagic ecosystems and description of a new model. Prog Oceanogr 71(1):1–33

Baumert HZ, Petzoldt T (2008) The role of temperature, cellular quota and nutrient concentrations for photosynthesis, growth and light-dark acclimation in phytoplankton. Limnologica 38:313–326

Beardall J, Burger-Wiersma T, Rijkeboer M, Sukenik A, Lemoalle J, Dubinsky Z, Fontvielle D (1994) Studies on enhanced post-illumination respiration in microalgae. J Plankton Res 16(10): 1401–1410

Becker S (2010) Biotic factors in induced defense revisited: cell aggregate formation in the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806 is triggered by spent *Daphnia* medium and disrupted cells. Hydrobiologia 644:159–168

Beckmann A, Hense I (2004) Torn between extremes: the ups and downs of phytoplankton. Ocean Dyn 54:581–592

Behrenfeld MJ, Halsey KH, Milligan AJ (2008) Evolved physiological responses of phytoplankton to their integrated growth environment. Philos Trans R Soc Lond B 363:2687–2703

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H (1998) *Chytridiomycosis* causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc Natl Acad Sci USA 95:9031–9036

Berman T (1997) Dissolved organic nitrogen utilization by an *Aphanizomenon* bloom in Lake Kinneret. J Plankton Res 19:577–586

Berman-Frank I, Lundgren P, Falkowski P (2003) Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Res Microbiol 154:157–164

Berman-Frank I, Bidle KD, Haramaty L, Falkowski PG (2004) The demise of the marine cyanobacterium, *Trichodesmium* spp., via an autocatalyzed cell death pathway. Limnol Oceanogr 49:997–1005

Berman-Frank I, Quigg A, Finkel ZV, Irwin AJ, Haramaty L (2007) Nitrogen-fixation strategies and Fe requirements in cyanobacteria. Limnol Oceanogr 52(5):2260–2269

Bhaya D, Schwarz R, Grossman AR (2000) Molecular responses to environmental stress. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 397–442, 669 pp

Bouchard JEN, Roy S, Campbell DA (2006) UVB effects on the photosystem II-D1 protein of phytoplankton and natural phytoplankton communities. Photochem Photobiol 82(4):936–951

Bowen CC, Jensen TE (1965) Blue-green algae: fine structure of the gas vacuoles. Science 147:1460–1462

Brookes JD, Carey CC (2011) Resilience to Blooms. Science 334:46–47

Brookes JD, Ganf GG, Burch MD (1994) The separation of forms of *Microcystis* from *Anabaena* in mixed populations by the application of pressure. Aust J Mar Freshw Res 4:133–138

Brookes JD, Ganf GG, Green D, Whittington J (1999) The influence of light and nutrients on buoyancy, filament aggregation and flotation of *Anabaena circinalis*. J Plankton Res 21:327–341

Brookes JD, Ganf GG, Oliver RL (2000) Heterogeneity of cyanobacterial gas vesicle volume and metabolic activity. J Plankton Res 22:1579–1589

Bruce LC, Hamilton DP, Imberger J, Gal G, Gophen M, Zohary T, Hambright KD (2006) A numerical simulation of the role of zooplankton in C, N and P cycling in Lake Kinneret, Israel. Ecol Model 193:412–436

Bruning K, Lingeman R, Ringelberg J (1992) Estimating the impact of fungal parasites on phytoplankton populations. Limnol Oceanogr 37:252–260

Brussaard CPD (2004) Viral control of phytoplankton populations – a review. J Eukaryot Microbiol 51:125–138

Bryant DA (1991) Cyanobacterial phycobilisomes: progress toward complete structural and functional analysis via molecular genetics. In: Bogorad L, Vasil IK (eds) Cell culture and somatic cell genetics of plants, vol 7B. Academic, New York, pp 255–298

Buranathanitt T, Cockrell DJ, John PH (1982) Some effects of Langmuir circulation on the quality of water resource systems. Ecol Model 15:49–74

Burger DF, Hamilton DP, Pilditch CA (2007) Modelling the relative importance of internal and external nutrient loads on water column nutrient concentrations and phytoplankton biomass in a shallow polymictic lake. Ecol Model 211:411–423

Burillo S, Luque I, Fuentes I, Contreras A (2004) Interactions between the nitrogen signal transduton protein PII and N-acetylglutamate kinase in organisms that perform oxygenic photosynthesis. J Bacteriol 186:3346–3354

Canter HM, Lund JWG (1968) The importance of protozoa in controlling the abundance of planktonic algae in lakes. Proc Linn Soc Lond 179:203–219

Caperon J, Meyer J (1972) Nitrogen-limited growth of marine phytoplankton – I. Changes in population characteristics with steady-state growth rate. Deep Sea Res 19:601–618

Carey CC, Ibelings BW, Hoffmann EP, Hamilton DP, Brookes JD (2012) Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate, Water Research doi:10.1016/j.watres.2011.12.016

Carmichael WW (1992) Cyanobacteria secondary metabolites – the cyanotoxins. J Appl Bacteriol 72:445–459

Carpenter SR (2008) Phosphorus control is critical to mitigating eutrophication. Proc Natl Acad Sci USA 105:11039–11040

Chu Z, Jin X, Yang B, Quigru Z (2007) Buoyancy regulation of *Microcystis flos-aquae* during phosphorus-limited and nitrogen-limited growth. J Plankton Res 29:739–745

Codd GA (1995) Cyanobacterial toxins: occurrence, properties and biological significance. Water Sci Technol 32(4):149–156

Codd GA, Steffensen DA, Burch MD, Baker PD (1994) Toxic blooms of cyanobacteria in Lake Alexandrina, South Australia – learning from history. Aust J Mar Freshw Res 45:1–6

Coruzzi G, Last R (2000) Amino acids. In: Buchannan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists Textbook, Rockville, Chapter 8, pp 358–410

Daft MJ, Stewart WDP (1971) Bacterial pathogens of freshwater blue-green algae. New Phytol 70:819–829

Damerval T, Castets A-M, Houmard J, Tandeau de Marsac N (1991) Gas vesicle synthesis in the cyanobacterium *Pseudanabaena* sp.: occurrence of a single photoregulated gene. Mol Microbiol 5:657–664

Davey MC, Walsby AE (1985) The form resistance of sinking algal chains. Br Phycol J 20:243–248
Deng L, Hayes PK (2008) Evidence for cyanophages active against bloom-forming freshwater cyanobacteria. Freshw Biol 53:1240–1252
Donnelly TH, Grace MR, Hart BT (1997) Algal blooms in the Darling-Barwon River Australia. Water Air Soil Pollut 99:487–496
Downing JA, Watson SB, McCauley E (2001) Predicting cyanobacteria dominance in lakes. Can J Fish Aquat Sci 58:1905–1908
Droop MR (1968) Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. J Mar Biol Assoc UK 48:689–733
Droop MR (1973) Some thoughts on nutrient limitation in algae. J Phycol 9:264–272
Elliott JA, Thackeray SJ, Huntingford C, Jones RG (2005) Combining a regional climate model with a phytoplankton community model to predict future changes in phytoplankton in lakes. Freshw Biol 50:1404–1411
Elliott JA, Elliot JA, Thackeray SJ (2006) Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. Hydrobiologia 559:401–411
Elser JJ (1999) The pathway to noxious cyanobacteria blooms in lakes: the food web as the final turn. Freshw Biol 42:537–543
Falconer IR, Beresford AM, Runnegar MTC (1983) Evidence of liver damage by toxin from a bloom of the blue-green alga *Microcystis aeruginosa*. Med J Aust 1:511–514
Falkowski PG, Kolber K (1993) Estimation of phytoplankton photosynthesis by active fluorescence. Int Counc Explor Sea Symp 197:92–103
Falkowski P, La Roche J (1991) Acclimation to spectral growth irradiance in algae. J Phycol 25:8–14
Faithful CL, Burns CW (2006) Effects of salinity and source of inocula on germination of Anabaena akinetes from a tidally influenced lake. Freshwat Biol 51:705–716
Ferguson AJD (1997) The role of modelling in the control of toxic blue-green algae. Hydrobiologia 349:1–4
Ferris JM, Christian R (1991) Aquatic primary production in relation to microalgal responses to changing light: a review. Aquat Sci 53:187–217
Flores E, Herrero A (2005) Nitrogen assimilation and nitrogen control in cyanobacteria. Biochem Soc Trans 33:164–167
Flynn KJ (2003a) Do we need complex mechanistic photoacclimation models for phytoplankton? Limnol Oceanogr 48(6):2243–2249
Flynn KJ (2003b) Modelling multi-nutrient interactions in phytoplankton; balancing simplicity and realism. Prog Oceanogr 56(2):249–279
Flynn KJ (2005) Castles built on sand: dysfunctionality in plankton models and the inadequacy of dialogue between biologists and modellers. J Plankton Res 27(12):1205–1210
Forchhammer K (2004) Global carbon/nitrogen control by P_{II} signal transduction in cyanobacteria: from signals to targets. FEMS Microbiol Rev 28:319–333
Forchhammer K (2008) P_{II} signal transducers: novel functional and structural insights. Trends Microbiol 16(2):65–72
Francis G (1878) Poisonous Australian lake. Nature (London) 18:11–12
Franklin DJ, Brussaard CPD, Berges JA (2006) What is the role and nature of programmed cell death in phytoplankton ecology. Eur J Phycol 41:1–14
Ganf GG, Oliver RL (1982) Vertical separation of light and available nutrients as a factor causing replacement of green algae by blue-green algae in the plankton of a stratified lake. J Ecol 70:829–844
Ganf GG, Oliver RL, Walsby AE (1989) Optical properties of gas-vacuolate cells and colonies of *Microcystis* in relation to light attenuation in a turbid, stratified reservoir (Mount Bold reservoir, South Australia). Aust J Mar Freshw Res 40:595–611
Garcia-Gonzalez M, Sivak MN, Guerrero MG, Preiss J, Lara C (1992) Depression of carbon flow to the glycogen pool induced by nitrogen assimilation in intact cells of *Anacystis nidulans*. Physiol Plant 86:360–364
Geider RJ, La Roche J (2002) Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. Eur J Phycol 37:1–17
Geider RJ, MacIntyre HL, Kana TM (1996) A dynamic model of photoadaptation in phytoplankton. Limnol Oceanogr 41:1–15
Geider RJ, MacIntyre HL, Kana TM (1998) A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. Limnol Oceanogr 43:679–694
Ghadouani A, Pinel-Alloul B, Prepas EE (2003) Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. Freshw Biol 48:363–381
Gibson CE (1978) Carbohydrate content as an ecological tool in the study of planktonic blue-green algae. Verh Int Ver Theor Angew Limnol 20:630–635
Glazer AN, Gindt YM, Chan CF, Sauer K (1994) Selective disruption of energy flow from phycobilisomes to photosystem I. Photosynth Res 40:167–173
Gons HJ, Hoogveld HL, Simis SGH, Tijdens M (2006) Dynamic modelling of viral impact on cyanobacterial populations in shallow lakes: implications of burst size. J Mar Biol Assoc UK 86:537–542
Gotham U, Rhee G-Y (1981) Comparative kinetic studies of nitrate-limited growth and nitrate uptake in phytoplankton in continuous culture. J Phycol 17:309–314
Grant NG, Walsby AE (1977) The contribution of photosynthate to turgor pressure rise in the planktonic blue-green alga *Anabaena flos-aquae*. J Exp Bot 28:409–415
Grossman A, Bhaya D, Apt K, Kehoe D (1995) Light-harvesting complexes in oxygenic photosynthesis: Diversity, control, and evolution. Ann Rev Genet 29:231–287
Guerrero MG, Lara C (1987) Assimilation of inorganic nitrogen. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 163–186, 543 pp
Guven B, Howard A (2006) A review and classification of the existing models of cyanobacteria. Prog Phys Geogr 30:1–24
Hamilton GS, Fielding F, Chiffings AW, Hart BT, Johnstone RW, Mengersen K (2007) Investigating the use of a Bayesian Network to model the risk of *Lyngbya majuscula* bloom initiation in Deception Bay, Queensland, Australia. Hum Ecol Risk Assess 13:1271–1287
Han B-P, Virtanen M, Koponen J, Straskraba M (2000) Effect of photoinhibition on algal photosynthesis: a dynamic model. J Plankton Res 22:865–885
Harris GP (1986) Phytoplankton ecology. Structure, function and fluctation. Chapman & Hall, London, 384 pp
Harris GP (1994) Pattern, process and prediction in aquatic ecology. A limnological view of some general ecological problems. Freshw Biol 32:143–160
Hayes PK, Walsby AE (1986) The inverse correlation between width and strength of gas vesicles in cyanobacteria. Br Phycol J 21:191–197
Healey FP (1978) Physiological indicators of nutrient deficiency in algae. Mitt Int Ver Theor Limnol 21:34–41
Healey FP (1982) Phosphate. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, pp 105–124, 676 pp
Hecky RE, Kilham P (1988) Nutrient limitation of phytoplankton in freshwater and marine environments; a review of recent evidence on the effects of enrichment. Limnol Oceanogr 33:796–822
Hecky RE, Campbell P, Hendzel LL (1993) The stoichiometry of carbon, nitrogen and phosphorus in particulate matter of lakes and oceans. Limnol Oceanogr 38:709–724
Heinrich A, Maheswaran M, Ruppert U, Forchhammer K (2004) The *Synechococcus elongatus* PII signal transduction protein controls arginine synthesis by complex formation with N-acetyl-l-glutamate kinase. Mol Microbiol 52(5):1303–1314
Hense I, Beckmann A (2006) Towards a model of cyanobacteria life cycle-effects of growing and resting stages on bloom formation of N_2-fixing species. Ecol Model 195(3–4):205–218
Hense I, Burchard H (2009) Modelling cyanobacteria in shallow coastal seas. Ecol Model 221(2):238–244

Heraud P, Beardall J (2000) Changes in chlorophyll fluorescence during exposure of *Dunaliella tertiolecta* to UV radiation indicate a dynamic interaction between damage and repair processes. Photosynth Res 63:123–134

Herrero A, Muro-Pastor AM, Flores E (2001) Nitrogen control in cyanobacteria. J Bacteriol 183:411–425

Hodges BR, Imberger J (2001) Simple curvilinear method for numerical methods of open channels. J Hydraul Eng ASCE 127:949–958

Hodges BR, Imberger J, Saggio A, Winters KB (2000) Modelling basin-scale internal waves in a stratified lake. Limnol Oceanogr 45:1603–1620

Holl CM, Montoya JP (2005) Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph *Trichodesmium* (Cyanobacteria). J Phycol 41:1178–1183

Horne AJ, Galat DL (1985) Nitrogen fixation in an oligotrophic, saline desert lake: Pyramid Lake, Nevada. Limnol Oceanogr 30:1229–1239

Howard A, Irish AE, Reynolds CS (1996) A new simulation of cyanobacterial underwater movement (SCUM '96). J Plankton Res 18:1375–1385

Howarth RG, Butler T, Lunde K, Swaney D, Chu CR (1993) Turbulence and planktonic nitrogen fixation: a mesocosm experiment. Limnol Oceanogr 38:1696–1711

Howarth RW, Chan F, Marino R (1999) Do top-down and bottom-up controls interact to exclude nitrogen-fixing cyanobacteria from the plankton of estuaries: explorations with a simulation model. Biogeochemistry 46:203–231

Huisman J, Hulot FD (2005) Population dynamics of harmful cyanobacteria. Factors affecting species composition. In: Huisman J, Matthijs HCP, Visser PM (eds) Harmful cyanobacteria. Springer, Dordrecht, pp 143–176

Huisman J, Jonker RR, Zonneveld C, Weissing FJ (1999) Competition for light between phytoplankton species: experimental test of mechanistic theory. Ecology 80:211–222

Huisman J, Sharples J, Stroom J, Visser PM, Kardinaal WEA, Verspagen JMH, Sommeijer B (2004) Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology 85:2960–2970

Humphries SE, Lyne VD (1988) Cyanophyte blooms: the role of cell buoyancy. Limnol Oceanogr 33:79–81

Hutchinson PA, Webster IT (1994) On the distribution of blue-green algae in lakes: Wind-tunnel tank experiments. Limnol Oceanogr 39:374–382

Ibelings BW, Maberly SC (1998) Photoinhibition and the availability of inorganic carbon restrict photosynthesis by surface blooms of cyanobacteria. Limnol Oceanogr 43:408–419

Ibelings BW, Marijke V, Los HFJ, van der Molen DT, Mooij WM (2003) Fuzzy modeling of cyanobacterial surface waterblooms: validation with NOAA-AVHRR satellite images. Ecol Appl 13:1456–1472

Imberger J, Hamblin JF (1982) Dynamics of lakes, reservoirs, and cooling ponds. Annu Rev Fluid Mech 14:153–187

Imberger J, Patterson JC (1981) A dynamic reservoir simulation model-DYRESM 5. In: Fischer HB (ed) Transport models for inland and coastal waters. Academic Press, New York, pp 310–361

Imberger J, Patterson JC (1990) Physical limnology. In: Hutchinson JW, Wu T (eds) Advances in applied mechanics, vol 27. Academic, Boston, pp 303–475

Imboden D (2004) The motion of lake waters. In: O'Sullivan P, Reynolds CS (eds) The lakes handbook: limnology and limnetic ecology. Blackwell Science Ltd, Oxford, pp 115–152

Izaguirre G, Hwang CJ, Krasner SW, McGuire MJ (1982) Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. Appl Environ Microbiol 43:708–714

Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol Oceanogr 21:540–547

Jöhnk KD, Huisman J, Sharples J, Sommeijer B, Visser PM (2008) Summer heatwaves promote blooms of harmful cyanobacteria. Glob Change Biol 14(3):495–512

Kagami M, de Bruin A, Ibelings BW, van Donk E (2007) Parasitic chytrids: their effects on phytoplankton communities and food-web dynamics. Hydrobiologia 578:113–129

Kehr JC, Zilliges Y, Springer A, Disney MD, Ratner DD, Bouchier C, Seeberger PH, Tandeau de Marsac D, Dittmann E (2006) A mannan binding lectin is involved in cell–cell attachment in a toxic strain of *Microcystis aeruginosa*. Mol Microbiol 59:893–906

Kerfeld CA (2004) Structure and function of the water-soluble carotenoid-binding proteins of cyanobacteria. Photosynth Res 81(3):1573–1579

Kirk JTO (1983) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge, 401 pp

Kirk JTO (1991) Volume scattering function, average cosines, and the underwater light field. Limnol Oceanogr 36:455–467

Kirk JTO, Oliver RL (1995) Optical closure in an ultraturbid lake. J Geophys Res 100:13221–13225

Klausmeier CA, Litchman E, Daufresne T, Levin SA (2004) Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429:171–174

Klemer AR (1978) Nitrogen limitation of growth and gas vacuolation in *Oscillatoria rubescens*. Verh Int Ver Theor Angew Limnol 20:2293–2297

Klemer AR (1991) Effects of nutritional status on cyanobacterial buoyancy, blooms and dominance with special reference to inorganic carbon. Can J Bot 69:1133–1138

Klemer AR, Feuillade J, Feuillade M (1982) Cyanobacterial blooms: carbon and nitrogen limitation have opposite effects on the buoyancy of *Oscillatoria*. Science 215:1629–1631

Klemer AR, Hendzel LL, Findlay DL, Hedin RA, Mageau MT, Konopka A (1995) Carbon availability and the pattern of cyanobacterial dominance in enriched low-carbon lakes. J Phycol 31:735–744

Kolber Z, Falkowski PG (1993) Use of active fluorescence to estimate phytoplankton photosynthesis *in situ*. Limnol Oceanogr 38:1646–1665

Kolodny NH, Bauer D, Bryce K, Klucevsek K, Lane A, Medeiros L, Mercer W, Moin S, Park D, Petersen J, Wright J, Yuen C, Wolfson AJ, Allen M (2006) Effect of nitrogen source on cyanophycin synthesis in *Synechocystis* sp. strain PCC 6308. J Bacteriol 188:934–940

Konopka A, Schnur M (1980) Effect of light intensity on macromolecular synthesis in cyanobacteria. Microb Ecol 6:291–301

Konopka A, Kromkamp J, Mur LR (1987a) Regulation of gas vesicle content and buoyancy in light or phosphate-limited cultures of *Aphanizomenon flos-aquae* (Cyanophyta). J Phycol 23:70–78

Konopka A, Kromkamp JC, Mur LR (1987b) Buoyancy regulation in phosphate-limited cultures of *Microcystis aeruginosa*. FEMS Microbiol Ecol 45:135–142

Kosten S, Huszar VLM, Becares E, Costa LS, van Donk E, Hansson LA, Jeppesenk E, Kruk C, Lacerot G, Mazzeo N, De Meester L, Moss B, Lurling M, Noges T, Romo S, Scheffer M (2012) Warmer climates boost cyanobacterial dominance in shallow lakes. Glob Change Biol 18(1):118–126

Kromkamp J (1987) Formation and functional significance of storage products in cyanobacteria. N Z J Mar Freshw Res 21:457–465

Kromkamp JC, Mur LR (1984) Buoyant density changes in the cyanobacterium *Microcystis aeruginosa* due to changes in the cellular carbohydrate content. FEMS Microbiol Lett 25:105–109

Kromkamp JC, Walsby AE (1990) A computer model of buoyancy and vertical migration in cyanobacteria. J Plankton Res 12:161–183

Kromkamp J, Konopka A, Mur LR (1986) Buoyancy regulation in a strain of *Aphanizomenon flos-aquae* (Cyanophyceae): the importance of carbohydrate accumulation and gas vesicle collapse. J Gen Microbiol 132:2113–2121

Kromkamp J, Konopka A, Mur LR (1988) Buoyancy regulation in light-limited continuous cultures *Microcystis aeruginosa*. J Plankton Res 10:171–183

Kruk C, Huszar VM, Peeters ETHM, Bonilla S, Coasta L, Rling MLU, Reynolds CS, Scheffer M (2010) A morphological classification capturing functional variation in phytoplankton. Freshw Biol 55:614–627

Laanemets J, Lilover M-J, Raudsepp U, Autio R, Vahtera E, Lips I, Lips U (2006) A fuzzy logic model to describe the cyanobacteria *Nodularia spumigena* blooms in the Gulf of Finland, Baltic Sea. Hydrobiologia 554:31–45

Latifi A, Ruiz M, Zhang CC (2009) Oxidative stress in cyanobacteria. FEMS Microbiol Rev 33(2):258–278

Levine SN, Lewis WM (1987) A numerical model of nitrogen fixation and its application to Lake Valencia, Venezuela. Freshw Biol 17:265–274

Lewis WM (1978a) Dynamics and succession of the phytoplankton in a tropical lake: Lake Lanao, Philippines. J Ecol 66:849–880

Lewis WM (1978b) Analysis of succession in a succession in a tropical phytoplankton community and a new measure of succession rate. Am Nat 112:401–418

Litchman E (2003) Competition and coexistence of phytoplankton under fluctuating light: experiments with two cyanobacteria. Aquat Microb Ecol 31:241–248

Liu Y, Xie P, Chen F, Wu X (2005) Effect of combinations of the toxic cyanobacterium *Microcystis aeruginosa* PCC7820 and the green alga *Scenedesmus* on the experimental population of *Daphnia pulex*. Bull Environ Contam Toxicol 74:1186–1191

Llácer JL, Fita I, Rubio V (2008) Arginine and nitrogen storage. Curr Opin Struct Biol 18:673–681

Los FJ (2009) Eco-hydrodynamic modelling of primary production in coastal waters and lakes using BLOOM. PhD thesis, Wageningen University, Wageningen. Available at http://edepot.wur.nl/1249

Los FJ, Villars MT, Van der Tol MWM (2008) A 3-dimensional primary production model (BLOOM/GEM) and its application to the southern North Sea (coupled physical-chemical-ecological model). J Mar Syst 74:259–274

Lund JWG, Reynolds CS (1982) The development and operation of large limnetic enclosures in Blelham Tarn, English Lake District, and their contribution to phytoplankton ecology. Prog Phycol Res 1:1–65

Luo L, Hamilton D(P), Han B (2010) Estimation of total cloud cover from solar radiation observations at Lake Rotorua, New Zealand. Solar Energy 84(3):501–506

Luque I, Flores E, Herrero A (1994) Nitrate and nitrite transport in the cyanobacterium *Synechococcus* sp. PCC 7942 are mediated by the same permease. Biochim Biophys Acta 1184:296–298

Luque I, Vazquez-Bermudez MF, Paz-Yepes J, Flores E, Herrero A (2004) In vivo activity of the nitrogen control transcription factor NtcA is subjected to metabolic regulation in Synechococcus sp strain PCC 7942. Fems Microbiol Lett 236(1):47–52

MacIntyre S (1993) Vertical mixing in a shallow, eutrophic lake: possible consequences for the light climate of phytoplankton. Limnol Oceanogr 38:798–817

MacIntyre HL, Kana TM, Anning T, Geider RJ (2002) Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. J Phycol 38(1):17–38

Mackerras AH, De Chazal NM, Smith GD (1990a) Transient accumulations of cyanophycin in *Anabaena cylindrica* and *Synechocystis* 6308. J Gen Microbiol 136:2057–2065

Mackerras AH, Youens BN, Weir RC, Smith GD (1990b) Is cyanophycin involved in the integration of nitrogen and carbon metabolism in the cyanobacteria *Anabaena cylindrica* and *Gloeothece* grown on light/dark circles? J Gen Microbiol 136:2049–2056

Maheswaran M, Ziegler K, Lokau W, Hagemann M, Forchhammer K (2006) PII-Regulated arginine synthesis controls accumulation of cyanophycin in *Synechocystis* sp. Strain PCC 6803. J Bacteriol 188(7):2730–2734

Mann NH (2006) Phages of cyanobacteria. In: Calendar R (ed) The bacteriophages. Oxford University Press, New York, pp 517–532, 746 pp

McNown JS, Malaika J (1950) Effects of particle shape of settling velocity on low Reynolds numbers. Trans Am Geophys Union 31:74–82

Middelboe M, Jacquet S, Weinbauer M (2008) Viruses in freshwater ecosystems: an introduction to the exploration of viruses in new aquatic habitats. Freshw Biol 53:1069–1075

Mooji WM, Trolle D, Jeppesen E, Arhonditsis G, Belolipetsky PV, Chitamwebwa DBR, Degermendzhy AG, DeAngelis DL, De Senerpont Domis LN, Downing AS, Elliott JA, Fragoso CR Jr, Gaedke U, Genova SN, Gulati RD, Håkanson L, Hamilton DP, Hipsey MR, 't Hoen J, Hülsmann S, Los FJ, Makler-Pick V, Petzoldt T, Prokopkin IG, Rinke K, Schep SA, Tominaga K, Van Dam AA, Van Nes EH, Wells SA, Janse JH (2010) Challenges and opportunities for integrating lake ecosystem modelling approaches. Aquat Ecol 44(3):633–667

Mur LR (1983) Some aspects of the ecophysiology of cyanobacteria. Ann Microbiol 134B:61–72

Murray AG (1995) Phytoplankton exudation: exploitation of the microbial loop as a defence against algal viruses. J Plankton Res 17:1079–1094

O'Brien KR, Meyer DL, Waite AM, Ivey GN, Hamilton DP (2004) Disaggregation of *Microcystis aeruginosa* colonies under turbulent mixing: laboratory experiments in a grid-stirred tank. Hydrobiologia 519:143–152

Oberhaus L, Briand JF, Leboulanger C, Jacquet S, Humbert JF (2007) Comparative effects of the quality and quantity of light and temperature on the growth of *Planktothrix agardhii* and *P. rubescens*. J Phycol 43(6):1191–1199

Ochoa de Alda JAG, Tapia MI, Fabrice F, Llama MJ, Serra JL (1996) Changes in nitrogen source modify distribution of excitation energy in the cyanobacterium *Phormidium laminosum*. Physiol Plant 97:69–78

Ohki K (1999) A possible role of temperate phage in the regulation of *Trichodesmium* biomass. Bull Inst Oceanogr (Monaco) 19:287–291

Ohlendiek U, Stuhr A, Siegmund H (2000) Nitrogen fixation by diazotrophic cyanobacteria in the Baltic Sea and transfer of the newly fixed nitrogen to picoplankton organisms. J Mar Syst 25:213–219

Okada M, Aiba S (1983a) Simulation of water bloom in a eutrophic lake. II: reassessment of buoyancy, gas vacuole and turgor pressure of *Microcystis aeruginosa*. Water Res 17:877–882

Okada M, Aiba S (1983b) Simulation of water-bloom in a eutrophic lake. III: modeling the vertical migration and growth of *Microcystis aeruginosa*. Water Res 17:883–893

Oliver R (1990) Optical properties of waters in the Murray-Darling Basin, south-eastern Australia. Aust J Mar Freshw Res 41:581–601

Oliver RL (1994) Floating and sinking in gas-vacuolate cyanobacteria. J Phycol 30:161–173

Oliver RL, Ganf GG (2000) Freshwater blooms. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 149–194, 669 pp

Oliver RL, Walsby AE (1984) Direct evidence for the role of light-mediated gas vesicle collapse in the buoyancy regulation of *Anabaena flos-aquae* (cyanobacteria). Limnol Oceanogr 29:879–886

Oliver RL, Whittington J (1998) Using measurements of variable chlorophyll-a fluorescence to investigate the influence of water movement on the photochemistry of phytoplankton. In: Imberger J (ed) Physical processes in lakes and oceans, Coastal and Estuarine Studies, Vol 54,American Geophysical Union, Washington, DC, pp 517–534, 668pp

Oliver RL, Kinnear AJ, Ganf GG (1981) Measurements of cell density of three freshwater phytoplankters by density gradient centrifugation. Limnol Oceanogr 26:285–294

Oliver R, Whittington J, Lorenz Z, Webster I (2003) The influence of vertical mixing on the photoinhibition of variable chlorophyll *a*

fluorescence and its inclusion in a model of phytoplankton photosynthesis. J Plankton Res 25(9):1107–1129
Ormerod JG (1992) Physiology of the photosynthetic prokaryotes. In: Mann NH, Carr NG (eds) Photosynthetic prokaryotes. Plenum Press, New York, pp 93–120
Patterson JC, Hamilton DP, Ferris J (1994) Modelling of cyanobacterial blooms in the mixed layer of lakes and reservoirs. Aust J Mar Freshw Res 45:829–845
Pollard P, Young LM (2010) Lake viruses lyse cyanobacteria, *Cylindrospermopsis raciborskii*, enhances filamentous-host dispersal in Australia. Acta Oecol 36:114–119
Rabouille S, Staal M, Stal LJ, Soetaert K (2006) Modeling the dynamic regulation of nitrogen fixation in the cyanobacterium *Trichodesmium* sp. Appl Environ Microbiol 72(5):3217–3227
Raven JA (2006) Aquatic viruses: the emerging story. J Mar Biol Assoc UK 86:449–451
Recknagel F, Cetin L, Zhang B (2008) Process-based simulation library SALMO-OO for lake ecosystems. Part 1: object-oriented implementation and validation. Ecol Inform 3:170–180
Reichwaldt ES, Stibor H (2005) The impact of diel vertical migration of *Daphnia* on phytoplankton dynamics. Oecologia 146:50–56
Reynolds CS (1984) The ecology of freshwater phytoplankton. Cambridge Univ. Press, Cambridge, 384 pp
Reynolds CS (1987) Cyanobacterial water blooms. In: Callow JA (ed) Advances in botanical research, vol 13. Academic, London, pp 67–143
Reynolds CS (1990) Temporal scales of variability in pelagic environments and the response of phytoplankton. Freshw Biol 23:25–53
Reynolds CS (1993) Scales of disturbance and their roles in plankton ecology. Hydrobiologia 249:151–171
Reynolds CS, Walsby AE (1975) Water blooms. Biol Rev 50:437–481
Reynolds CS, Wiseman SW, Clarke MJO (1984) Growth- and loss-rate responses of phytoplankton to intermittent artificial mixing and their potential application to the control of planktonic algal biomass. J Appl Ecol 21:11–39
Reynolds CS, Oliver RL, Walsby AE (1987) Cyanobacterial dominance: the role of buoyancy regulation in the billowing environment. N Z J Mar Freshw Res 2:379–390
Reynolds CS, Irish AE, Elliott JA (2001) The ecological basis for simulating phytoplankton responses to environmental change (PROTECH). Ecol Model 140(3):271–291
Richardson K, Beardell J, Raven JA (1983) Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phycol 93:157–191
Riegman R, Mur LC (1984) Regulation of phosphate uptake kinetics in *Oscillatoria agardhii*. Arch Microbiol 139:28–32
Robson BJ, Hamilton DP (2004) Three-dimensional modelling of a *Microcystis* bloom event in the Swan River estuary. Ecol Model 174:203–222
Romans KM, Carpenter EJ, Bergman B (1994) Buoyancy regulation in the colonial diazotrophic cyanobacterium *Trichodesmium tenue*: ultrastructure and storage of carbohydrate, polyphosphate and nitrogen. J Phycol 30:935–942
Ross ON, Geider RJ (2009) New cell-based model of photosynthesis and photo-acclimation: accumulation and mobilisation of energy reserves in phytoplankton. Mar Ecol Prog Ser 383:53–71
Ross ON, Sharples J (2004) Recipe for 1-D Langrangian particle tracking models in space-varying diffusivity. Limnol Oceanogr Method 2:289–302
Ross C, Santiago-Vazquez L, Paul V (2006) Toxin release in response to oxidative stress and programmed cell death in the cyanobacterium *Microcystis aeruginosa*. Aquat Toxicol 78:66–73
Sakamoto M (1966) Primary production by phytoplankton community in some Japanese lakes and its dependence upon lake depth. Arch Hydrobiol 62:1–28
Sathyendranath S, Platt T, Forget MH (2007) Oceanic primary production: comparison of models. Oceans 2007 – Europe international conference, Aberdeen, Scotland, IEEE
Sauer J, Schreiber U, Schmid R,Volker U, Forchhammer K (2001). Nitrogen starvation-induced chlorosis in Synechococcus PCC 7942. Low-level photosynthesis as a mechanism of long-term survival. Plant Physiol 126:233 –243
Scheffer M, Rinaldi S, Gragnani A, Mur LR, van Ness EH (1997) On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology 78:72–282
Schindler DW (1971) Carbon, nitrogen and phosphorus and the eutrophication of freshwater lakes. J Phycol 7:321–329
Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, Beaty KG, Lyng M, Kasian SEM (2008) Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. Proc Natl Acad Sci USA 105(32):11254–11258
Schopf JW, Packer BM (1987) Early Archean (3.3 billion to 3.5 billion-year-old) microfossils from Warrawoona Group, Australia. Science 237:70–73
Schreiber U, Endo T, Mi H, Asada K (1995) Quenching analysis of chlorophyll fluorescence by the saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. Plant Cell Physiol 36:873–882
Schwarz R, Forchhammer K (2005) Acclimation of unicellular cyanobacteria to macronutrient deficiency: emergence of a complex network of cellular responses. Microbiology 151:2503–2514
Scott JT, McCarthy MJ (2010) Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. Limnol Oceanogr 55(3):1265–1270
Sedmak B, Elersek T (2006) Microcystins induce morphological and physiological changes in selected representative phytoplanktons. Microb Ecol 51:508–515
Segovia M, Haramaty L, Berges JA, Falkowski PG (2003) Cell death in the unicellular chlorophyte *Dunaliella tertiolecta*: an hypothesis on the evolution of apoptosis in higher plants and metazoans. Plant Physiol 132:99–105
Shilo M (1970) Lysis of blue-green algae by myxobacter. J Bacteriol 104:453–461
Shilo M (1971) Biological agents that cause lysis of blue-green algae. Mitt Int Ver Theor Limnol 19:206–213
Shuter B (1979) A model of physiological adaptation in unicellular algae. J Theor Biol 78(4):519–552
Sigee DC, Selwyn A, Gallois P, Dean AP (2007) Patterns of cell death in freshwater colonial cyanobacteria during the late summer bloom. Phycologia 46:284–292
Simon RD (1987) Inclusion bodies in the cyanobacteria: cyanophycin, polyphosphate, polyhedral bodies. In: Fay P, van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam, pp 199–225
Singh AK, Elvitigala T, Bhattacharyya-Pakrasi M, Aurora R, Ghosh B, Pakrasi HB (2008) Integration of carbon and nitrogen metabolism with energy production is crucial to light acclimation in the cyanobacterium *Synechocystis*. Plant Physiol 148(1):467–478
Singh AK, Bhattacharyya-Pakrasi M, Elvitigala T, Ghosh B, Aurora R, Pakrasi HB (2009) A systems-level analysis of the effects of light quality on the metabolism of a cyanobacterium. Plant Physiol 151:1596–1608
Smith VH (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green in lake phytoplankton. Science 221:669–671
Sommer U, Sommer F, Santer B, Zellner E, Jergens K, Jamieson C, Boersma M, Gocke K (2003) *Daphnia* versus copepod impact on summer phytoplankton: functional compensation at both trophic levels. Oecologia 135:639–647
Stal LJ (2009) Is the distribution of nitrogen-fixing cyanobacteria in the oceans related to temperature? Environ Microbiol 11:1632–1645
Stal LJ, Walsby AE (1998) The daily integral of nitrogen fixation by planktonic cyanobacteria in the Baltic Sea. New Phytol 139:665–671
Stal LJ, Walsby AE (2000) Photosynthesis and nitrogen fixation in a cyanobacterial in the Baltic Sea. Eur J Phycol 35:97–108

Steinberg CEW, Hartmann HM (1988) Planktonic bloom-forming cyanobacteria and the eutrophication of lakes and rivers. Freshw Biol 20:279–287

Stomp M, Huisman J, de Jongh F, Veraart AJ, Gerla D, Rijkeboer M, Ibelings BW, Wollenzien UIA, Stal LJ (2004) Adaptive divergence in pigment composition promotes phytoplankton biodiversity. Nature 432(7013):104–107

Stomp M, Huisman J, Stal LJ, Matthijs HCP (2007) Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. ISME J 1(4):271–282

Stone S, Ganf G (1981) The influence of previous light history on the respiration of four species of freshwater phytoplankton. Arch Hydrobiol 91:435–462

Takano K, Ishikawa Y, Mikami H, Igarashi S, Hino S, Yoshioka T (2008) Fungal infection for cyanobacterium *Anabaena smithii* by two chytrids in eutrophic region of large reservoir Lake Shumarinai, Hokkaido, Japan. Limnology 9:213–218

Tandeau de Marsac N, Houmard J (1993) Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol Rev 104:119–190

Tandeau de Marsac N, Lee HM, Hisbergues M, Castets AM, Bédu S (2001) Control of nitrogen and carbon metabolism in cyanobacteria. J Appl Phycol 13:287–394

Tapia MI, Ochoa de Alda JAG, Llama MJ, Serra JL (1996) Changes in intracellular amino acids and organic acids induced by nitrogen starvation and nitrate or ammonium resupply in the cyanobacterium *Phormidium laminosum*. Planta 198:526–531

Thompson PA, Waite AM, McMahon K (2003) Dynamics of a cyanobacterial bloom in a hypereutrophic, stratified weir pool. Mar Freshw Res 54:27–37

Tilman D, Kilham SS, Kilham P (1982) Phytoplankton community ecology: the role of limiting nutrients. Annu Rev Ecol Syst 13:349–372

Trimbee AM, Prepas EE (1987) Evaluation of total phosphorus as a predictor of the relative biomass of blue-green algae with emphasis on Alberta lakes. Can J Fish Aquat Sci 14:1337–1342

Trolle D, Hamilton DP, Pilditch CA, Duggan IC (2010) Predicting the effects of climate change on trophic status of three morphologically varying lakes: implications for lake restoration and management. Environ Model Softw 26:354–370

Tucker S, Pollard P (2005) Identification of cyanophage Ma-LBP and infection of the cyanobacterium *Microcystis aeruginosa* from an Australian subtropical lake by the virus. Appl Environ Microbiol 71:629–635

Turpin DH (1991) Effects of inorganic N availability on algal photosynthesis and carbon metabolism. J Phycol 27:14–20

Utkilen HC, Oliver RL, Walsby AE (1985) Buoyancy regulation in a red *Ocillatoria* unable to collapse gas vacuoles by turgor pressure. Arch Hydrobiol 102:319–329

van Rijn J, Shilo M (1985) Carbohydrate fluctuations, gas vacuolation, and vertical migration of scum-forming cyanobacteria in fishponds. Limnol Oceanogr 30(6):1219–1228

Vázquez-Bermúdez MF, Herrero A, Flores E (2003) Carbon supply and 2-oxoglutarate effects on expression of nitrate reductase and nitrogen-regulated genes in *Synechococcus* sp. strain PCC 7942. FEMS Microbiol Lett 221(2):155–159

Venkatesh MM, Maidment DR, Hodges BR (2005) Geospatial representation of river channels. J Hydrol Eng ASCE 10:243–251

Verspagen JMH, Snelder EOFM, Visser PM, Huisman J, Mur LR, Ibelings BW (2004) Recruitment of benthic *Microcystis* (Cyanophyceae) to the water column: internal buoyancy changes or resuspension? J Phycol 40:260–270

Vincent WF (2009) Cyanobacteria. In: Likens GE (ed) Encyclopedia of inland waters, vol 3. Elsevier, Oxford, pp 226–232

Visser PM, Ibelings BW, Mur LR (1994) Autumnal sedimentation of *Microcystis* spp. as result of an increase in carbohydrate ballast at reduced temperature. J Plankton Res 17:919–933

Visser PM, Ibelings BW, Van Der Veer B, Koedoods J, Mur LR (1996) Artificial mixing prevents nuisance blooms of the cyanobacterium *Microcystis* in Lake Nieuwe Meer, The Netherlands. Freshw Biol 36:435–450

Visser PM, Passarge J, Mur LR (1997) Modelling vertical migration of the cyanobacteria *Microcystis*. Hydrobiologia 349:99–109

Vollenweider RA (1968) Scientific fundamentals of the eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Technical report DA 5/SCI/68.27. OECD, Paris, 250 pp

Wallace BB, Hamilton DP (1999) The effect of variations in irradiance on buoyancy regulation in *Microcystis aeruginosa*. Limnol Oceanogr 44:273–281

Wallace BB, Hamilton DP (2000) Simulation of water-bloom formation in the cyanobacterium Microcystis aeruginosa. J Plankton Res 22(6):1127–1138

Wallace BB, Bailey MC, Hamilton DP (2000) Simulation of vertical position of buoyancy regulating *Microcystis aeruginosa* in a shallow eutrophic lake. Aquat Sci 62:320–333

Walsby AE (1971) The pressure relationships of gas vacuoles. Proc R Soc Lond B 178:301–326

Walsby AE (1985) The permeability of heterocysts to the gases nitrogen and oxygen. Proc R Soc Lond B 226:345–366

Walsby AE (1987) Mechanisms of buoyancy regulation by planktonic cyanobacteria with gas vesicles. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier Science Publishers, Amsterdam, pp 377–392, 543 pp

Walsby AE (1994) Gas vesicles. Microbiol Rev 58:94–144

Walsby AE (2007) Cyanobacterial heterocysts: terminal pores proposed as sites of gas exchange. Trends Microbiol 15:340–349

Walsby AE, Bleything A (1988) The dimensions of cyanobacterial gas vesicles in relation to their efficiency in providing buoyancy and withstanding pressure. J Gen Microbiol 134:2635–2645

Walsby AE, Reynolds CS (1980) Sinking and floating. In: Morris I (ed) The physiological ecology of phytoplankton. Blackwell Scientific Publications, Oxford, pp 371–412

Walsby AE, Reynolds CS, Oliver RL, Kromkamp J, Gibbs MM (1987) The role of buoyancy in the distribution of *Anabaena* sp. in Lake Rotongaio. N Z J Mar Freshw Res 21:525–526

Walsby AE, Reynolds CS, Oliver RL, Kromkamp J (1989) The role of gas vacuoles and carbohydrate content in the buoyancy and vertical distribution of *Anabaena minutissima* in Lake Rotongaio, New Zealand. Arch Hydrobiol Ergeb Limnol 32:1–25

Walsby AE, Kinsman R, Ibelings BW, Reynolds CS (1991) Highly buoyant colonies of the cyanobacterium *Anabaena lemmermannii* form persistent surface waterblooms. Arch Hydobiol 121:261–280

Walsby AE, Hayes PK, Boje R, Stal LJ (1997) The selective advantage of buoyancy provided by gas vesicles for planktonic cyanobacteria in the Baltic Sea. New Phytol 136:407–417

Walsby AE, Dubinsky Z, Kromkamp JC, Lehmann C, Schanz F (2001) The effects of diel changes in photosynthetic coefficients and depth of *Planktothrix rubescens* on the daily integral of photosynthesis in Lake Zürich. Aquat Sci 63:326–349

Webster IT, Hutchinson PA (1994) Effects of wind on the distribution of phytoplankton cells in lakes-revisited. Limnol Oceanogr 39:365–373

Wilhelm S, Matteson AR (2008) Freshwater and marine virioplankton: a brief overview of commonalities and differences. Freshw Biol 53:1076–1089

Wood SA, Prentice MJ, Smith K, Hamilton DP (2010) Low dissolved inorganic nitrogen and increased heterocyte frequency: precursors to *Anabaena planktonica* blooms in a temperate, eutrophic reservoir. J Plankton Res 32:1315–1325

Wyman M, Fay P (1986) Underwater light climate and the growth and pigmentation of planktonic blue-green algae (Cyanobacteria) I. the influence of light quality. Proc R Soc Lond B 227:381–393

Yang Z, Kong F, Shi X, Cao H (2006) Morphological response of *Microcystis aeruginosa* to grazing by different sorts of zooplankton. Hydrobiologia 563:225–230

Yang Z, Kong F, Shi X, Zhang M, Xing P, Cao H (2008) Changes in the morphology and polysaccharide content of *Microcystis aeruginosa* (Cyanobacteria) during flagellate grazing. J Phycol 44:716–720

Zevenboom W, Mur LR (1978) On nitrate uptake by *Oscillatoria agardhii*. Verh Int Ver Limnol 20:2302–2307

Zhao J, Ramin M, Cheng V, Arhonditsis B (2008) Competition patterns among phytoplankton functional groups: how useful are the complex mathematical models? Acta Oecol 33(3): 324–344

Zilliges Y, Kehr JC, Mikkat S, Bouchier C, Tandeau N, de Marsac T, Borner E, Dittmann E (2008) An extracellular glycoprotein is implicated in cell-cell contacts in the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806. J Bacteriol 190:2871–2879

Microcystis

7

Lenka Šejnohová and Blahoslav Maršálek

Contents

L. Šejnohová
Institute of Botany, Academy of Sciences of the Czech Republic,
Lidická 25/27, Brno CZ-65720, Czech Republic
e-mail: eustigmatos@gmail.com

B. Maršálek (✉)
Centre for Cyanobacteria and their Toxins, Institute of Botany,
Academy of Sciences of the Czech Republic,
Lidická 25/27, Brno CZ-65720, Czech Republic
e-mail: marsalek@sinice.cz

Summary

The chapter focuses on features of *Microcystis* influencing its success in forming water blooms world-wide. The topics covered included its life strategy, life cycle, cell structure and function, together with the environmental variables especially important at different stages in its life cycle. A polyphasic approach to its taxonomy has shown the complexity of the situation in nature and in the laboratory. While the genus is distinct enough to be recognized, we suggest using "morphospecies" for descriptions of species. The increasing literature on the benthic phase of the life cycle in temperate regions is reviewed, together with the subsequent reinvasion of colonies to the plankton. Temperature and bioperturbation appear to be among the most important factors influencing the latter stage and there is no evidence for any sort of time clock. There has been a shift during recent years from considering extracellular and intracellular peptides, alkaloids and other biologically active compounds largely with respect to their human impact, especially toxicity, to a broader one of understanding their ecological role. Partly associated with this the process of colony formation has become an important area of study. Morphological, ecophysiological approaches combined with molecular genetic and sensitive instrumental methods can open a new view on signal transduction and intercellular communication within individual colonies and whole populations. Such information will not only aid the understanding of colony formation, but also

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bloom formation, populations dynamic and the competitive advantages of various "morphospecies".

7.1 Introduction

The mass development of planktonic cyanobacteria forming blooms causes problems in many nutrient-rich waterbodies around the world and, among the organisms involved, *Microcystis* is perhaps the most notorious (Fig. 7.1). The environmental factors which have been suggested to lead to cyanobacterial dominance in such waters include low light, elevated water temperature, high pH/low CO_2 and TN/TP ratio, while important features of the organisms themselves include buoyancy control, storage strategy at the bottom of the water column, inorganic nitrogen strategy, higher requirements for some trace elements than eukaryotic phytoplankton and resistance to zooplankton grazing (Varis 1993; Hyenstrand et al. 1998; Huisman and Hulot 2005; Onderka 2007). This reviews sets out to assess which of these environmental factors and organism features are especially important for *Microcystis*. Some information on other plankton genera is included where it helps to make the situation with *Microcystis* clear.

Blooms of *Microcystis* develop in standing and slow-moving freshwaters all over the world and also in marine coastal waters (Parra et al. 1980) apart, apparently, from the polar regions (Komárek and Komárková 2002; Chap. 14). Standing waters with *Microcystis* range from small ponds to some of the largest lakes and are characterized by the stability of their water column for part of the year (Reynolds et al. 1981; Bonnet and Poulin 2002; Visser et al. 2005). Interest in the genus has increased with the eutrophication of many freshwaters and the problems associated with increases in *Microcystis* growths. The odours, off-flavour compounds and various toxic substances produced by *Microcystis* can significantly impair drinking water quality and hence have a huge economic impact (Falconer 2005).

Microcystis is well known as a producer of hepatotoxins, especially microcystins, which can decrease the abundance and richness of macro-invertebrate communities (White et al. 2005) and poison domestic livestock and wild animals (Oberholster et al. 2009; Chap. 24); microcystins are also one of the factors involved in fish kills during blooms. However, there is no clear evidence for magnification of microcystins within food webs so far (Ibelings et al. 2005).

Microcystis has also been shown to produce a large variety of other bioactive oligopeptides (Fastner et al. 2001; Welker et al. 2006; Welker and von Döhren 2006), so it not only their toxicity to warm-blooded organisms, but their ecological roles, such as the communication within populations, which are becoming important for consideration (Orr and Jones 1998; Sedmak and Elersek 2005; Schatz et al. 2007).

The typical annual cycle of *Microcystis* in temperate regions, where the organism has received the most study, includes the development in summer of blooms in the plankton, followed by the population sinking to the bottom in autumn, overwintering on or in the upper layers of sediment and reinvasion into the water column in spring. The colonies persisting on sediment under conditions unfavourable for growth (Brunberg 1995) can even form a biomass substantially in excess of the maximum in the plankton (Bostrom et al. 1989). Vertical migration depends on external factors causing changes in buoyancy due especially to the density of gas vesicles (Thomas and Walsby 1985b), but also proteins and carbohydrates (Kromkamp and Mur 1984; Visser et al. 2005).

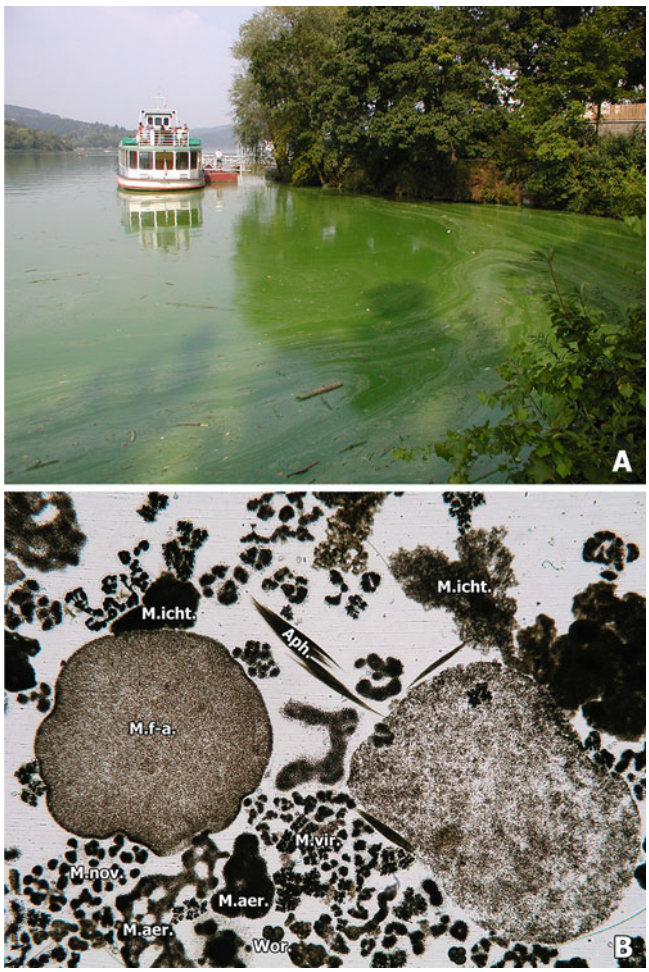

Fig. 7.1 Surface bloom of *Microcystis* on Brno Reservoir, Czech Republic, August 2006: (**a**) General view; (**b**) Plankton sample, showing individual *Microcystis* morphospecies (abbrev. see below), *Aphanizomenon klebahnii* (Aph) and *Woronichinia naegeliana* (Wor)

7.2 Morphology and Taxonomy

The variety of names used to record the range of *Microcystis* forms found in nature can make it hard to compare the results of different studies, so this account starts with comments on their taxonomy. As with other groups of cyanobacteria, modern taxonomic classification requires a combination of

markers, with molecular data correlated with biochemical, ultrastructural, phenotypic and ecological data (Komárek 2010). The genus *Microcystis* appears to be well delimited as shown by molecular markers (Lyra et al. 2001; Janse et al. 2003; Sanchis et al. 2005) and chemotaxonomy (Gugger et al. 2002), as well as morphological characters. The genus is defined by its spherical cells irregularly grouped into colonies with or without colourless, stratified, mucilage. Formally, it belongs to the order Chroococcales and the family Microcystaceae Elenkin 1933. The colonies range from micro- to macroscopic and also differ morphologically during the vegetative cycle. Increase in colony numbers depends on separation of parts of the original colony or disintegration of the whole colony. Cell division occurs in three planes perpendicular one to another in successive generations and the daughter cells are almost hemispherical immediately after division. The resting cells never differentiate into walled structures. Gas vesicles are formed in at least in one part of the vegetative cycle (Komárek and Anagnostidis 1998; Anagnostidis and Komárek 1985).

Traditional *Microcystis* "species" have been described according to colony form, mucilage structure, cell diameter, the density and organization of cells within the colony, pigment content (phycocyanin:phycoerythrin ratio) and life cycles (Komárek and Komárková 2002). The term morphospecies has become widely used for species recognized on such morphological criteria. The variability of colonies is, however, very wide and many populations overlap the limits usually accepted for a particular morphospecies (Otsuka et al. 2000). Colony size of *M. aeruginosa* and some, but not all other bloom-forming cyanobacteria studied by Yamamoto et al. (2011) increased with increasing dominance of the species in the plankton. Material presenting a challenge for naming is especially likely to occur at the beginning or the end of a particular vegetative period. Culture studies are not a reliable aid, because differences occur between morphospecies in the extent to which cell size in culture corresponds to that in nature, as found for a comparison between *M. aeruginosa, M. viridis* and *M. wesenbergii* (Komárková et al. 2005); other authors have failed to show significant differences between *M. flos-aquae* and *M. ichthyoblabe* in nature and the laboratory. More than 50 morphospecies of *Microcystis*have been described, with 20 in temperate regions and at least 11 in Europe (Komárek and Anagnostidis 1998; Komárek and Komárková 2002). It is evident that the taxonomy needs revision (see www.cyanodb.cz/microcystis).

Studies of *Microcystis* taxonomy using molecular and biochemical criteria have provided results which sometimes appear contradictory and so caution is needed in their interpretation. 16S rRNA analysis revealed no differences among morphospecies (Otsuka et al. 1998, 2001). In contrast, a study based on allozyme divergence suggested that *M. viridis* and *M. wesenbergii* are well-established species (Kato et al. 1991). Similar conclusions were found during the analysis of the diversity of microcystins: *M. wesenbergii* was in all cases non-microcystin producing, separating it clearly from other morphospecies (Kurmayer et al. 2002; Via-Ordorika et al. 2004). A comparison of a wide spectrum of oligopeptides distinguished three *Microcystis* groups: *M. aeruginosa* with microcystins and aeruginosins, *M. ichthyoblabe* with anabaenopeptins and microginins (Fastner et al. 2001; Šejnohová et al. 2011); *M. wesenbergii* with cyanopeptolins and unknown peptides (Fastner et al. 2001). An assessment (Sanchis et al. 2005) based on both 16S rRNA–23S rRNA ITS and cpcBA-IGB regions suggests a monophyletic identity for *M. novacekii* and *M. wesenbergii* and a close relationship between *M. novacekii* and *M. aeruginosa.* Chemotyping based on peptide pattern (Welker et al. 2007) showed that *M. wesenbergii* did not produce any peptide, *M. viridis* is specified by microcystin-cyanopeptolin chemotype and the three other morphospecies tested (*M. aeruginosa*, *M. novacekii*, *M. ichthyoblabe*) were represented by several chemotypes. 16S rRNA gene sequences of five strains of *M. smithii* isolated from Lake Dishui, China, intermixed with strains of other morphospecies at three different positions in a phylogenetic tree (Liu et al. 2011). These facts demonstrate that the "species" category in *Microcystis* is problematic and doubtful. Nevertheless, traditional morphospecies with distinct phenotypic and ecophysiological features are still needed for ecological and ecotoxicological studies. Consequently, the taxonomic unification of all the main morphospecies (*M. aeruginosa, M. ichthyoblabe*, *M. viridis*, *M. novacekii*, *M. wesenbergii*) suggested by Otsuka et al. (2001) seems premature until the reasons of their physiological and morphological diversity can be explained (Komárek and Komarková 2002). It remains unclear why the classification of *Microcystis* under the rules of the International Code of Bacteriological Nomenclature should differ from any other classification (Otsuka et al. 2001).

In conclusion, the above results and our own research indicate that there are at least three clusters within *Microcystis* defined by a combination of morphological and molecular markers. These are: small (S) cell-size group close to *M. ichthyoblabe* (incl. *M. flos-aquae)*, middle (M) cell-size group based on *M. aeruginosa* (incl. *M. novacekii*); large (L) cell-size group represented by *M. wesenbergii* (Fig. 7.2, Table 7.1). The status of *M. viridis*, which appears closely related to the *M. aeruginosa* group, needs further research.

7.3 Cell Structure and Function

7.3.1 Cell and Cell Wall

The ultrastructure of *Microcystis* cells has been described for various morphospecies using material from cultures (Jost and Zehnder 1966; Jost and Jones 1970) and nature

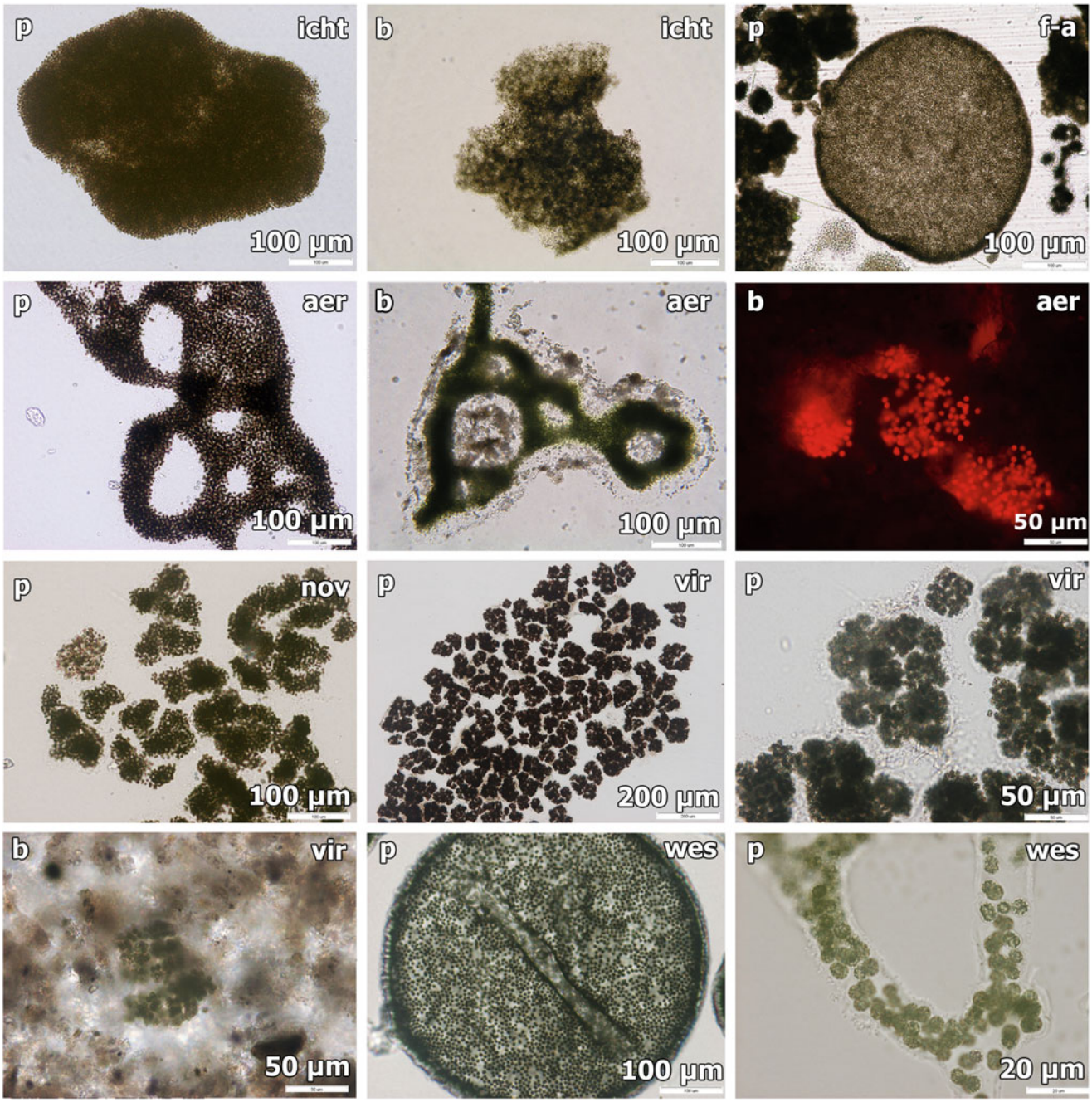

Fig. 7.2 The main morphospecies of *Microcystis* in Europe: *M. ichthyoblabe* (icht), *M. flos-aquae* (f-a), *M. aeruginosa* (aer), *M. novacekii* (nov), *M. viridis* (vir), *M. wesenbergii* (wes); *p* plankton, *b* benthic. *Dark colour* of colonies is due to the high number of gas vesicles, which are retractile thus it appears *black* under light microscope (Photo by L. Šejnohová)

(Belikova 1978; Reynolds et al. 1981). The cell wall surface is surrounded by polysaccharide mucilage with associated heterotrophic bacteria in natural populations (Brunberg and Bostrom 1992). The structure of the cell inside the wall is quite similar for all morphospecies, though slight differences were reported during the annual growth cycle of *M. aeruginosa* (Reynolds et al. 1981). The differences between morphospecies in the structure of the cell wall S-layer, which has a variety of roles in bacteria, such as a protective coat, molecular sieve and molecule and ion traps, promoter for cell adhesion and surface recognition and determining and maintaining cell shape or envelope rigidity, was shown with the freeze-fracture technique (Sleytr et al. 1988). The S-layer protein comprises up to 10% total cell protein (Šmarda 1991). In the case of *Microcystis*, the proteinaceous surface may be influenced by environmental conditions (Šmajs and Šmarda 1999), so it remains unclear whether there really are differences between morphospecies.

Table 7.1 The main and common European *Microcystis* morphospecies (Komárek and Komárková 2002; Šejnohová 2008)

Cell-size cluster µm	Morphospecies	Cell diameter	Form of colony	Cell arrangement	Mucilage type	size µm
S (small) 2–4.8	***ichthyoblabe***	2–3.2	Irregular	Densely regularly agglomerated	Indistinct	0
	flos-aquae	3.5–4.8	Spherical	Densely regularly agglomerated	Diffuse, slightly overlapping cells	5
M (medium) (3.5)4.5–6(7)	***aeruginosa***	4–6.5	Irregular	Densely regularly agglomerated	Diffuse, slightly overlapping cells	25
	novacekii	(2.4)3–6	Irregular	Not densely irregularly with lobates with cells in clusters	Diffuse, slightly overlapping cells	15
	viridis	4–7.9	Irregular	Not dense irregular with clearly cubic arrangement	Distinct, clearly overlapping cells	5–(10)
L (large) 6–8.5	***wesenbergii***	(4)6–8.5	Irregular	Not densely irregularly with lobates	Distinct, distant, clearly delimited, with refractive margin	2

Morphospecies shown in bold letters are the main morphospecies for the cluster

7.3.2 Gas Vesicles

7.3.2.1 Introduction

One of the diagnostic features of *Microcystis* is the presence of gas vesicles (GV, Fig. 7.3). Their main function is to enable the buoyancy of the cell to respond to changing environmental conditions in the water column (Reynolds and Walsby 1975). Gas vesicles, originally termed gas vacuoles (Anagnostidis 1961), are made up of hollow gas-filled structures with conical end caps, which occur in certain prokaryotes from aquatic habitats (Walsby 1972). *Microcystis* was among the first four genera in which GV were reported, the others being *Gloeotrichia*, *Aphanizomenon* and *Nostoc* (Klebahn 1895; Ahlborn 1895). The discovery that "gas vacuoles" consist of numerous, cylindrical gas vesicles which are integrated parallel in bundles (so-called "aerotopes"), was first reported in the halophilic bacterium *Halobacterium halobium* (Houwink 1956), but a few years later were shown to be similar in the cyanobacterium *Aphanizomenon* (Bowen and Jensen 1965). Many studies then followed on their ultrastructure in *Microcystis*, initially in culture (Jost and Zehnder 1966; Smith and Peat 1967; Jones and Jost 1970), but subsequently also field samples (Belikova 1978; Reynolds et al. 1981). These studies were included in the comprehensive review by Walsby (1994). Two theories about the role in addition to buoyancy regulation, gas storage (Klebahn 1922; Kolkwitz 1928) have long been rejected.

7.3.2.2 Structure and Chemistry of Gas Vesicle Membrane

Under light microscopy gas vesicles are observed as aerotopes which can be distinguished from other granules by their bright and refractile qualities, with a slight reddish appearance (Walsby 1972). Electron microcopy of gas vesicles membranes has showed an unusual composition, being main protein and no lipid (Stoeckenius and Kunau 1968). The membrane is three-layered and thinner than the typical

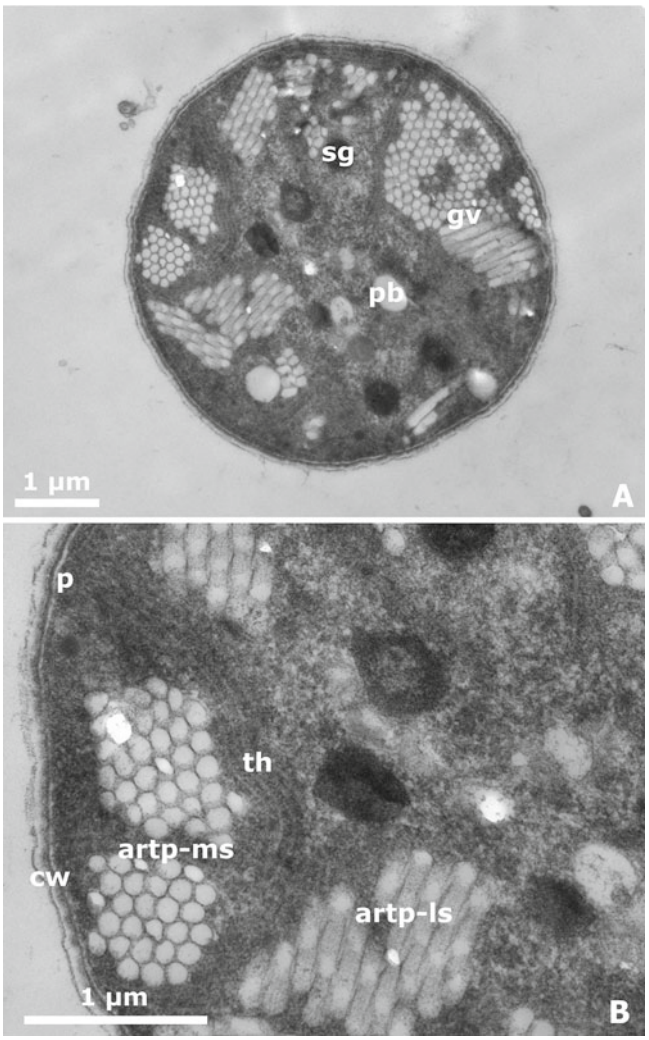

Fig. 7.3 Ultrastructure of *Microcystis* cells in sediment before reinvasion in February: (**a**) Median section of vegetative cell, low number of gas vesicles (*gv*) and higher amount of storage products polyphosphate body (*pb*), carboxysomes (*c*). (**b**) Detail of quadrilayered cell wall (*cw*), plasmalemma (*p*), thylakoids (*th*) and gas vesicles (Photo by O. Benada and L. Šejnohová)

cell unit membrane. Whereas the gas vesicles membrane is 8 nm wide in *Halobacterium* (Stoeckenius and Rowen 1967), in *Microcystis* it is only 3 nm (Jones and Jost 1970). There are at least two types of GV membrane proteins in *Microcystis*:

GvpA – the main hydrophobic small protein arranged in a linear crystalline array along ribs. This protein forms the hollow shell of GV. It is a main component of GV in all cyanobacteria with gas vesicles and must be responsible for many of GV properties (Walsby 1994).

GvpC – the large hydrophilic protein on the outer surface, which is a minor component. Its function is strengthening the GV, without this protein GV are weaker and collapse at a much lower critical pressure (Walsby and Hayes 1988).

7.3.2.3 Morphology of Gas Vesicles

A GV grows from a small biconical structure, which is formed by a complicated process of protein assembling encoding by genes in nucleoplasm. The biconical initials enlarge GV to a critical diameter and then they grow longer by extension of the cylinder formed between the terminal cones (Waaland and Branton 1969). It is collapsed due to high pressure, a new GV is formed in the same way but cannot be rebuilt directly from the collapsed GV. They are largely synthesized *de novo*, with recycling of some of the protein from destroyed membranes (Hayes and Walsby 1984). The production of GV is very fast; in *Microcystis* a GV may reach to its maximum length of 600 nm in 12 h.

The GV in all cyanobacteria have the same basic morphology – hollow cylinders with conical ends, but they vary in dimensions. Length also varies widely within the one cell, ranging from 0.1 μm to more than 1 μm, but the diameter is more or less constant (Šmajs et al. 1999). The width is fairly specific for each "species", but it can depend partly on surrounding conditions and is controlled genetically by natural selection (Walsby 1994). *Microcystis* from culture and from freshwater reservoir has narrower GV (65 nm) than *Planktothrix* (Šmarda and Šmajs 1996) and *Anabaena* (Walsby and Bleything 1988). The critical collapse pressure of GV should be inversely related to their width (Walsby 1971). Wider GV are more fragile and than narrower ones, but wider GV provide buoyancy with the highest efficiency. Narrower GV are to be anticipated in organisms subject to high osmotic pressure. The orientation of GV to form parallel bundles may increase gas permeability in the cell (Walsby 1994).

7.3.2.4 Content and Physical Properties of Gas Vesicles

As GV are gas-filled and their membrane is freely permeable to small gas molecules, the internal gas composition is equal to that of the water surrounding the cell. GV are permeable only to small molecules such as H_2, N_2, O_2, CO_2, CO, CH_4 and Ar, because the pore diameter in the membrane is 0.36 nm. Liquid cannot seep into the GV, because the inner surface of the membrane is hydrophobic (Walsby 1969). The time required for equilibration of gas across the wall of GV is only in milliseconds or less and dependent on the actual pressure (Walsby 1984). The diffusion of gases inside the GV is 1,000 time quicker than through a lipid-monolayer (Walsby 1994). The surface of GV is constantly exposed to pressure, the net pressure being the total outer pressure minus the gas pressure inside the GV. If the outer pressure rises, this is transmitted immediately to GV through the suspending water and the GV may then collapse (Walsby 1994). The gas from a collapsed GV diffuses away rather than escaping as a bubble (Walsby 1971).

In the study by Walsby and Bleything (1988) mentioned above, the medium critical pressure for the *Microcystis* populations was higher (0.65–1.10 MPa) than in the populations of the two other bloom-forming genera (*Anabaena*, *Aphanizomenon* 0.60 MPa). In a study by Rueter and Petersen (1987), the critical pressure necessary to collapse the GV in *Microcystis* ranged from 6 to 11 bar with the median critical pressure (causing 50% collapse) occurring at 7.5 bar (0.75 MPa). The turgor pressure increased as soon as the light period started, reaching a maximum in about 1–2 h (Thomas and Walsby 1985b). Simultaneously, the collapse-pressure was affected by the rate of pressure rise. Under a rapid, almost instantaneous, rise, there was a larger initial decrease in turgor. Following collapse of half the gas vesicles, the cells recovered their full turgor pressure after 3 h. This suggests turgor homeostasis (Holland and Walsby 2009).

7.3.3 Intracellular Granules and Buoyancy

Like other cyanobacteria, intracellular granules mostly contain reserve substances, the main ones in *Microcystis* being shown in Fig. 7.3. Glycogen granules, which are the first visible product of photosynthesis (Lang 1968), are often in close association with regions of the thylakoids and are irregular bodies about 16×33 nm. Cyanophycin granules store N and consist of a co-polymer of aspartate and arginine, which are essential components in the synthesis of gas vesicles. During N starvation they are degraded (Oliver and Ganf 2000). Cyanophycin is accumulated when cells have a plentiful N supply, but other environmental factors such as light, P or S are inadequate to permit this N to be utilized in further cell metabolic processes, including growth at low temperatures (Allen 1984). These granules are slightly angular and can be seen with light microscope with staining (Lockau and Ziegler 2006). Although phycocyanin is a major pigment component of the light-harvesting antenna, it also functions as a N reserve, condition of nitrogen limitation it acts as a nitrogen reserve. N starvation leads to them being degraded and the cells them become bleached (Tandeau de

Marsac and Houmard 1993). When the cells are exposed to a N supply, phycocyanin synthesis occurs after the that of cyanophycin (Allen 1984).

Other structures include those with polyphosphate, polyhydroxybuturate or RuBisCO Polyphosphate bodies (volutin) provide P storage. *Microcystis* has a high V_{max} for phosphate uptake, a low minimum P content and a large capacity to accumulate P (Kromkamp et al. 1989a) and hence importance of polyphosphate bodies. Their size in *Microcystis* ranges from 100 to 400 nm diameter (Jacobson and Halmann 1982). Accumulation of toluidine blue (metaphosphate stain, Ebel 1952) can aid their recognition by light microscopy. The polyphosphate bodies can contain various metals, with potassium, calcium, magnesium and some heavy-metals being reported in the study by Kromkamp (1987). Poly-β-hydroxybutyric acid (PHB) is a lipid polymer containing structures about 200 nm diameter and often abundant in *Microcystis* cells. A feature distinguishing these structures from polyphosphate is the 3-nm limiting monolayer (Reynolds et al. 1981). Carboxysomes contain the RuBisCO which catalyses photosynthetic fixation of CO_2. When the CO_2 concentration is low, their number rises (Oliver and Ganf 2000).

Nutrient uptake controls the amount of nutrients inside the cell which are transferred to the granules. Besides a storage function some of these granules can influence sinking during conditions of energy stress (Kromkamp 1987). For instance, the buoyant density changes due to variations in the cellular carbohydrate content (Kromkamp and Mur 1984). *M. aeruginosa* cells of 5 μm diameter were found to accumulate carbohydrate of density 1,600 kg m^{-3} (Kromkamp and Walsby 1990). Although buoyancy is regulated mainly by accumulation of carbohydrate in response to the light, polyphosphate may also affect cell buoyancy (Brookes and Ganf 2001), whereas ribosomes and small proteins have little influence (Walsby 1980). During N-limitation, GV volume per cell decreased (to 84–88%) markedly with N exhaustion, while the carbohydrate content accumulated. During P-limitation, GV volume per cell decreased slightly (maximum to 22–32%), and the GV still provided sufficient buoyancy i.e. N limitation caused a more significant buoyancy loss than P limitation. Long-term buoyancy regulation was determined mainly by changes in GV volume, whereas short-term buoyancy regulation was determined mainly by carbohydrate accumulation and consumption. Long-term and short-term buoyancy regulation are both influenced by cell nutrient status (Chu et al. 2007b).

7.3.4 Genome and Genes

M. aeruginosa NIES-843 has a single, circular chromosome of 5,842,795 base-pairs with an average GC content of 42.3% (Kaneko et al. 2007). The putative protein-encoding sequences show 45% sequence similarity to genes of known function and 32% to hypothetical genes, while the remaining 23% have no apparent similarity to reported genes. In addition to known gene clusters related to the synthesis of microcystin and cyanopeptolin, novel gene clusters that may be involved in the synthesis and modification of toxic small polypeptides have been identified.

The most detailed gene studies on *Microcystis* have been conducted on those encoding GV synthesis (Mlouka et al. 2004a). According to Min et al. (2007), there are *gvpA* and *gvp*C structural genes encoding gas vesicle proteins with high variability of the *gvpA-gvpC* region, which should be useful in identifying geographical isolates or ecotypes. The diameter of the GV correlates positively with the number of amino acid residue repeats in *gvpC* gene. The number of sequences of *gvpC* between two *Microcystis* strains may differ by 1–5% (Dunton and Walsby 2005). Culture experiments have shown that *Microcystis aeruginosa* under standard growth conditions can form GV-deficient mutants, which cannot synthesize GV proteins, although the mutant may have *gvpA* encoding the major GV protein. Inactivation of the *gvp* genes causes insertion sequences (IS), which are mobile DNA elements (Mlouka et al. 2004b). The loss of *gvp* genes can be spontaneous, leading to non-buoyant mutants (Beard et al. 2002).

7.4 Ecological Variables

7.4.1 Light

Reynolds et al. (1981) suggested that the occurrence of *Microcystis* blooms is affected by the interaction between light attenuation and the stability of the water column synergistically with high nutrient loadings (but low N:P ratio), raised pH and decreased CO_2, tolerance of low O_2 concentration and low redox potential, which affect the availability of sulphur, iron and other metals, and the resistance of colonies to grazing. The comprehensive model by Huisman et al. (1999a) identified four key parameters for bloom development: incident light intensity, background low turbidity, water column depth and turbulent mixing rates. This computer model also predicts that the turbulent mixing rate is a major determinant of the species composition of phytoplankton blooms. Field results support this, with light, temperature, nutrient concentration, turbidity, and wind speed and direction all being factors which affected *Microcystis* spp. density (Wang et al. 2009). This subject is considered in more detail in Chap. 6.

7.4.1.1 Critical Depth and Stratification

In aquatic environments a prime factor contributing to environmental heterogeneity is the vertical light gradient which

is a function of depth, dissolved compounds, particles and at least partly is also created by the phototrophic organisms themselves; hence changes in species composition are usually accompanied by changes in the vertical shading pattern (Huisman et al. 1999a, c). The multispecies model predicts that well-mixed waters favour species with the lowest critical light intensity, or equivalently, the species with deepest critical depth. Incomplete mixing, in contrast, favours species that are able to obtain the best vertical positions within a water column, such as by gas vesicles providing buoyancy. The first direct demonstration that light intensity influenced the buoyancy of planktonic cyanobacteria was provided by Walsby (1969). Accordingly, the species composition in well-mixed waters should generally differ from that in waters of low turbulence (Huisman et al. 1999b). The fact that *Microcystis* can form huge biomasses in stratified nutrient-rich lakes means that light availability and its converse, turbidity, have a crucial role in buoyancy regulation of the colonies in both diurnal and annual cycles (Bonnet and Poulin 2002; Visser et al. 2005). The dominating genera *Microcystis*, *Aphanizomenon* and *Anabaena* achieved their highest biomass where stratified conditions exceed a duration of 3 weeks.

7.4.1.2 Light Intensity

Increasing the light intensity eventually leads to photosynthetic activity reaching Pmax, while further increases lead to a decline in activity (Yagi et al. 1994). Because of the influence of light on buoyancy, *Microcystis* colonies from the surface layers become less buoyant during the day and more so at night (Konopka et al. 1978; Ibelings et al. 1991; Oliver 1994). This vertical movement effectively regulates the amount of incident light on thylakoid and photosynthetic activity (Oliver and Walsby 1984). As buoyancy is influenced not only by light, but also the previous nutrient history of the cell (Sect. 7.3.3; Brookes and Ganf 2001), the response to light can be influenced by the nutrient status. For instance, upon relief from P limitation, the buoyancy of *Microcystis* cultures increased whether the cultures were incubated in the light or dark (Oliver 1994; Kromkamp et al. 1989b).

Several studies on the buoyancy of *Microcystis* in culture have investigated in detail the role of carbohydrate in counteracting the buoyancy provided by GV (Thomas and Walsby 1985a; Visser et al. 2005). Synthesis of a high polysaccharide level starts when the *Microcystis* is transferred from light-limited conditions to high light intensities (Kromkamp and Mur 1984). Because of the accumulation of products such as carbohydrates, the sedimentation rate increases in the afternoon (Visser et al. 1996b). This process of polysaccharide formation is reversible: when light is reduced, the polysaccharide content decreases in nutrient-limited cultures (Kromkamp 1987). During light limitation, GV tend to accumulate and their formation is dependent on the available energy reserves (Deacon and Walsby 1990; Kromkamp 1987). *M. aeruginosa* incubated in the dark following exposure to light formed more GV if their previous irradiation had been high and they had accumulated carbohydrate (Brookes and Ganf 2001). Low light may also causes changes in the pigment content of *Microcystis* cells (Zevenboom and Mur 1984).

The optimal irradiance for GV formation by *Microcystis* was found to be over 20 µmol photon m^{-2} s^{-1} and reached a maximum from 30 (Deacon and Walsby 1990) to 60 µmol photon m^{-2} s^{-1} (Kromkamp et al. 1989a, b). Constant high irradiance (90 µmol photon m^{-2} s^{-1}) led to a decrease in number of thylakoids and an increase in PHB, lipids bodies, the thickness of peptidoglycan layer, whereas cyanophycin granules were only scarce in the mutant culture without GV in 25°C after 12 h. After 30 days the intracellular reserves (PHB, glycogen granules) completely filled the cells, thylakoids were scarce and the culture was bleached. Floating occurred in GV-free cultures because of their contents of PHB and lipid droplets (Canini et al. 2003).

In another set of experiments, cells in culture exposed to high irradiance lost buoyancy due to the accumulation of carbohydrates, which overcame the lift produced by GV (Thomas and Walsby 1985b). Wallace and Hamilton (1999) also simulated changing light conditions by increasing radiation up to 1,300 µmol photon m^{-2} s^{-1}, the value they had measured at noon in the field; and showed that the *de novo* synthesis of carbohydrates caused sinking of the colonies to the sediment. In nature this mechanism applies in surface layers of blooms when the cells are exposed to full sunlight and high photoirradiance may induce photoinhibition (Ibelings 1996). At noon *Microcystis* showed a higher value for photoinhibition (up to 41%) than chlorophytes (32%) and diatoms/dinoflagellates (34%) at the surface. No significant difference in diurnal growth rates among the three phytoplankton groups was observed, indicating that *Microcystis* could photoacclimate comparably with the eukaryotic algae. In addition, *Microcystis* had a higher non-photochemical quenching value than the others at the surface at noon, which suggests that cyanobacteria may be better at dissipating excess energy (Zhang et al. 2008a).

Microcystis synthesizes mycosporine-like amino acids (MAAs) and carotenoids to cope with high solar UV radiation at the water surface. The maximum MAA concentration per cell observed (2.5% dry weight) will confer only ~40% of internal screening to a single layer of *Microcystis* cells. Other strategies include the screening of UV radiation by d-galacturonic acid, one of the main chemical components of the mucilage layer in *Microcystis.* Thus, the formation of a colony with several layers of cells is important to afford an efficient UV screening by internal self-shading (Sommaruga et al. 2009). *M. aeruginosa* has at least three adaptive strategies to cope with the enhanced UV-B: increasing the synthesis of carotenoids to counteract reactive oxidants caused by UV-B

exposure, degrading phycocyanin and allophycocyanin to avoid further damage to DNA and reaction centres, and enhancing the repair of UV-B induced damage to the photosynthetic apparatus (Jiang and Qiu 2005). Other cellular responses to reduce the susceptibility of the cyanobacterial membranes to photo-oxidation include changes in the fatty acid composition (Walsh et al. 1997, 1998). Further details about responses to UV-radiation are described in Chap. 19.

7.4.1.3 Photoperiod

During growth with a short photoperiod (8 h) cyanobacteria are able to sustain relatively high growth rates and the loss of polysaccharide is minimized by decreased respiration (Kromkamp 1987). In *M. aeruginosa* cultures growth in a L:D cycle with a constant content of phosphate, diurnal fluctuations in buoyancy were associated only with the carbohydrate content, the GV density remaining constant (Kromkamp et al. 1988). Light dependent formation of GV proteins was observed by *M. aeruginosa* after a phosphate pulse in a P-starved culture under a photon flux density of 50 µmol m^{-2} s^{-1}; GV formation did not occur in the dark. The increase in volume of the gas vesicles is correlated with the length of the light period (Kromkamp et al. 1989b). When a buoyant culture was transferred from a photon flux density of 15–200 µmol photon m^{-2} s^{-1}, the turgor increased from 2.5 to 4.3 bar, but this was not enough for GV collapse (Kromkamp et al. 1988). Tolerance of *M. aeruginosa* at 25°C to the dark was weak, with biomass decreasing to only 1% of initial value after 20 days. In contrast, *Scenedesmus* (green alga) and *Melosira* (centric diatom) retained their biomass after even 20 days. After restarting the L:D cycle, however, *Microcystis* increased exponentially and reached maximum biomass levels similar to *Scenedesmus* and *Melosira* (Furusato et al. 2004).

7.4.2 Wind

Webster (1990) presented a theory showing how the circulation and mixing induced by a steady wind blowing above a lake should lead to horizontal heterogeneity in the distribution of buoyant phytoplankton population in the lake. The vertical distribution of phytoplankton and stability of the water column can be strongly affected by the wind, with coefficients of variation of pigment concentrations throughout the water column showing a negative correlation with increasing wind and waves (Cao et al. 2006). When a lake is a strongly mixed by wind, all suspended phytoplankton are stirred through the epilimnion. Small-celled phytoplankton will be incapable of moving up the water column, but buoyant *Microcystis* colonies float up into the higher irradiance near the water surface (Visser et al. 2005). Surface proportions of *M. aeruginosa* versus total amounts in the water column also closely correlated with wind and waves, which demonstrated that wind and waves directly influenced surface *M. aeruginosa* blooms (Cao et al. 2006). Small colonies of *Microcystis* are easily affected by wind-induced mixing. In contrast, large colonies tend to show little diurnal repositioning and are thus mainly concentrated in the surface layer (Wu and Kong 2009). Experimental evidence suggests that winds having speeds >2–3 m s^{-1} are required to mix floating phytoplankton cells (or colonies) away from the water surface (Webster and Hutchinson 1994; Webster 1990). If the surface wind speed is greater than 8 m s^{-1}, sediment is intensively suspended in shallow waterbodies and littoral zones. The dynamic effects of the wind-induced wave disturbance produced by a strong wind may be the main mode for internal release of total P, total dissolved P, dissolved reactive P and algae available phosphorus (AAP) from sediments into overlying water. Strong winds have important effects on the nutrient supply during a *Microcystis* bloom (Zhu et al. 2005). According to model predictions with a lake experiment, *Microcystis* dominated at low turbulent diffusivity, whereas sinking diatoms and green algae dominated at high turbulent diffusivity (Huisman et al. 2004).

7.4.3 Temperature

Water temperature is one of the critical factors for *Microcystis* development during the whole annual cycle (Reynolds et al. 1981; Wu et al. 2010). Temperature is especially important for buoyancy regulation – synthesis of gas vesicles, rate of photosynthesis and carbohydrate production. High temperatures increase the stability of the water column, thereby reducing vertical turbulent mixing, which shifts the competitive balance in favour of buoyant cyanobacteria (Caceres and Reynolds 1984; John et al. 2008). In a study by Robarts and Zohary (1987), the optimal temperature for photosynthesis of a New Zealand *Microcystis* population was 20°C, the same as for *Anabaena* and *Aphanizomenon*. However, a decrease to 15°C led to the photosynthetic rate of *Microcystis* from Lake Mendota (Wisconsin) falling to 4% maximum, in contrast to *Anabaena* and *Aphanizomenon* with rates of 76% and 48%, respectively (Konopka and Brock 1978). According to Varis (1993), researching in Finland, the optimum temperature for maximum development of *Microcystis* is in the range of 19–25°C, whereas it is lower for *Anabaena* and *Aphanizomenon* (15–20°C). Hammer (1964) reported mass development of *Microcystis* colonies in the range 17.5–26°C with the optimum at 20°C. A study (Yagi et al. 1994) of three Japanese *Microcystis* strains, each given a different specific name, found optimum photosynthetic activity for at 30°C for *M. aeruginosa* and *M. wesenbergii*, but at 25°C for *M. viridis*. Comparison by Wu et al. (2010) of the temperature responses of *M. aeruginosa* and *Anabaena flos-aquae* in Dianchi Lake,

China, showed that while the former failed to grow at 10°C and only very slowly at 15°C, it grew faster than *A. flos-aquae* at 20°C and 25°C, suggesting that temperature may be the dominating factor in its success.

Several other studies have investigated the effects of temperature on competition between *Microcystis* and other organisms, both cyanobacteria and eukaryotes. Chu et al. (2007a), using lake simulator systems (microcosms), showed that *Oscillatoria mougeotii* outcompeted *Microcystis aeruginosa* at <20°C, whereas the converse occurred at 30°C. *O. mougeotii* had a long exponential phase (20 days) and a low growth rate of 0.22 and 0.20 day^{-1} at 15°C and 20°C, respectively, whereas *M. aeruginosa* had a shorter exponential phase (2–3 days) at 30°C and a higher growth rate 0.86 day^{-1}. Above the optimum temperature there was a sharp decline in photosynthetic activity, although this ceased altogether only between 45°C and 50°C. Based on laboratory studies, Fujimoto et al. (1994) concluded that *Microcystis* is able to adapt more quickly to temperature fluctuations than green algae. This applied to *M. viridis* and *Selenastrum capricornutum* in a study in the range 14–30°C.

The study by Furusato et al. (2004), which involved growth of *Microcystis aeruginosa* under a L:D cycle, found that floating cells occurred during the light period in light-limited cultures at 20°C and 28°C, whereas at 15°C buoyancy was lost at the start of light period. However, cultures grown under high irradiance (50 µmol photon m^{-2} s^{-1}) during the light period lost buoyancy at both 8°C and 20°C due to accumulation of carbohydrate (Furusato et al. 2004). These results fit with the conclusion that the maximum rate of photosynthesis, specific respiration rate and growth rate are all temperature independent in light-limited *Microcystis* cultures, whereas in light-saturated cultures these characteristics are temperature dependent (Preston et al. 1980; Thomas and Walsby 1986). As mentioned above, low temperature causes a buoyancy loss by an increase in polysaccharide (decrease respiration rate of polysaccharide) and reduction in GV volume.

In temperate climates *Microcystis* seems to be a genus which develops when the water temperature rises later in the growth season as the water temperature rises (Robarts and Zohary 1987). This fits with the reports that *Microcystis* mass development is limited by low temperatures more severely than other genera of bloom-forming cyanobacteria, with sharp declines in growth rate below a critical temperature of about 15°C (Kromkamp et al. 1989b). However, there are examples, where *Microcystis* biomass persisted in the water column during the whole winter. This has occurred, for instance in the shallow Czech reservoir Nové Mlýny, where the temperature was 5–8°C and the density was 120–200,000 cells mL^{-1}, and also in several Dutch shallow lakes (authors, unpublished data).

Water temperature changes during summer may be an important factor influencing the alternating succession of *Microcystis* morphospecies. In a study by Imai et al. (2009) carried out in Furuike Pond, Japan, *M. aeruginosa* was more abundant than *M. wesenbergii* in June and August to early October, while *M. wesenbergii* was more abundant in July and between late October and November. The water temperature during the period when *M. aeruginosa* was dominant was higher (24.7–33.9°C) than that when *M. wesenbergii* was (19.6–28.6°C). In laboratory experiments the growth rates of *M. aeruginosa* isolates were significantly higher than those of *M. wesenbergii* at high temperatures (30°C and 35°C). However, the growth rates of the two species were similar at lower temperatures (20°C and 25°C). Strong correlations between temperature and *Microcystis* operational taxonomic units (OTUs) composition have been reported using canonical correspondence analysis (CCA). A phylogenetic tree based on the sequencing results of target bands on 16S–23S rRNA internal transcribed spacer using denaturing gradient gel electrophoresis (DGGE) indicated that samples collected in summer and winter constituted two separate clusters (Tan et al. 2009).

Temperature is also important for *Microcystis* populations in sediments, where they persist as vegetative cells (Brunberg and Bostrom 1992). In Russia and Ukraine, for instance, the optimum conditions for overwintering were found to be 4–5°C (Guseva 1952; Sirenko 1972). Temperature is especially important during renewed activity in the spring (Caceres and Reynolds 1984).

7.4.4 Dissolved Oxygen and Water Mixing

In what was probably the first detailed ecological study of *Microcystis* it was concluded that the viability and recruitment of colonies is supported by anoxic conditions on the bottom layers of water (Guseva 1952). Anoxic and dark conditions caused a slight increase in cell metabolic activity, no conspicuous cell death, and no decay of chlorophyll-a fluorescence for *M. aeruginosa*. In contrast, cell metabolic activity and fluorescence of *Scenedesmus obliquus* decreased sharply, and cell concentrations fluctuated markedly with time. *Microcystis aeruginosa* appeared to be more tolerant to dark anoxic conditions than *Scenedesmus obliquus*, conditions likely to arise in eutrophic lakes beneath thick surface scums in the water column, or in the bottom sediments. Tolerance of these conditions may be important for the dominance of *M. aeruginosa* in eutrophic lakes (Shi et al. 2007). Conditions where there was an increase in redox potential in sediments rich in hydrogen sulphide were associated with the process of oxidation by free radicals, with the effect of reducing cell viability. Thus, *Microcystis* dominated mainly in low-flow reservoirs rich in organic matter (Sirenko 1972). Subsequent studies have confirmed this negative correlation of mass development of blooms with high oxygen saturation and provided information on the effect on particular phases of the *Microcystis* annual cycle.

The recruitment of colonies into the main waterbody is initiated by several factors, such as high surface temperature and light penetration to the bottom, but the crucial factor influencing different stages is the oxygen concentration, whether the development of a low concentration (Caceres and Reynolds 1984; Reynolds et al. 1981; Trimbee and Harris 1984; Trimbee and Prepas 1988) or anoxic conditions (Konopka and Brock 1978; Oliver et al. 1985). Thus, hypolimnetic aeration is one of the possible techniques for reducing the number of benthic *Microcystis* colonies, because of its effect on chemical and microbial processes and consequent reduced release of P, Fe, Mn and H_2S from the sediments. However, such management may disrupt thermal layers important to the fish community and even promote supersaturation of water with gases harmful to fish (Caceres and Reynolds 1984; Trimbee and Prepas 1988). Aeration is widely used to mix shallow lakes (circulation) and sometimes to destratify deep lakes. Artificial mixing has been adopted specifically to prevent *Microcystis* blooms (Holdren et al. 2001; Chen et al. 2009a) and decreased *Microcystis* biomass due to artificial mixing has been observed by many authors (Toetz 1981; Visser et al. 1996a; Lindenschmidt 1999). Diatoms and green algae may in fact profit from artificial mixing, as this reduces their sedimentation losses and may lower the pH and thus shift the inorganic carbon complex towards CO_2 (Visser et al. 2005). One of the earliest aeration systems was developed in Lake Bret, Switzerland (Mercier and Perret 1949). The effects of turbulence have been reported from various lakes, such as Lake Nieuwe Meer, The Netherlands. However, artificial mixing is less efficient than aeration and is not always successful in reducing the cyanobacterial biomass (Visser et al. 2005). Deppe et al. (1999) showed that a control strategy, which combined P reduction with the transport of hypolimnetic water rich in free CO_2 to the epilimnion, completely suppressed *Microcystis.* When aeration was accompanied by light-shading, the efficiency in reducing algal biomass increased with an increase in residence time (Chen et al. 2009a). With a residence time of 5 days, a combination of light-shading and aeration, led to a reduction in biomass of more than 65%. As *Microcystis* tends to float upwards under light-limited conditions, an integrated system consisting of a pre-separation stage and light-shading plus aeration treatment was suggested to treat dense populations. This study realized in China showed that pre-separation could remove more than 40% of cyanobacterial biomass, and the total reduction efficiency of the integrated system increased to above 80%.

7.4.5 Nutrients

7.4.5.1 Overview

The ideal quotas relative to the ash-free dry mass of healthy, growing cells are 50% carbon, 8.5% nitrogen and 1.5% phosphorus by mass, these elements occurring in approximately the ratios 41C:7N:1P (i.e. C:N = 1:6), although in nature this may differ between different species. Based on atomic weights of the elements (≈12, 14, 31) and normalizing to P, the molecular ratio for a healthy biomass is 106C:16N:1P (Reynolds 2006). This ratio is generally referred to as the Redfield ratio (Redfield 1958). Stumm and Morgan (1996) extended the ideal stochiometric representation of protoplasmic composition to the other major components (>1% of ash-free dry mass – hydrogen, oxygen, sulphur) or some others that frequently limit phytoplankton growth in nature (silicon, iron). To date, there is a huge literature on this topic. The purpose here is not to review the findings in detail, but to show how the key elements influence the ability of *Microcystis* to gather the resources necessary to support cell growth and replication.

Laboratory experiments have demonstrated that growth of cyanobacteria can be described as a function of the concentration of the limiting nutrient, especially P and N. The uptake rate (V) of the limiting nutrient into the cells can be described by the Michaelis-Menten as a function of the external nutrient concentration (s):

$$V = V_{\max} s / (K_m + s)$$

where Km is half-saturation constant for uptake

During steady-state growth the uptake rate of a nutrient is dependent upon the internal content according to:

$$q = \mu Q$$

where: q is steady-state uptake rate; μ is growth rate; Q is internal nutrient content

In general, micro-organisms respond to a limiting nutrient concentration by increasing V_{max} (Rhee 1980; Visser et al. 1996a). A sudden increase in available nutrients leads to a rapid accumulation of nutrients in the cells and the cells respond by decreasing the uptake rate up towards inhibition, which is defined by the inhibition constant (k). This inhibition kinetics can be of ecological significance because species with a low inhibition constant can store more phosphate than species with a high k. In a study with *Microcystis* the value for k was 0.8 h. compared with 1.1 h for *Oscillatoria* (Kromkamp et al. 1989a). Such attributes suggest *Microcystis* is a storage specialist (Kilham and Hecky 1988).

7.4.5.2 Phosphorus

Phosphorus is widely accepted as the main nutrient controlling the development of natural populations of cyanobacteria in many freshwater environments. A field investigation (Wu et al. 2010) of the seasonal dynamics of *M. aeruginosa* indicated that total P, total N and transparency are the next most important correlative factors influencing its succession, the most important being temperature. The main sources of P for *Microcystis* species are orthophosphates, but they can also use organic forms. Moreover, the growth rate correlates

with the availability of P (Reynolds et al. 1981). Usually it requires less than 0.03 mg L^{-1} P to be sufficient to permit cyanobacteria mass development.

As mentioned in Sect. 7.3.3, *Microcystis* can accumulate P in polyphosphate bodies (volutin granules) (Jacobson and Halmann 1982; Allen 1984). This strategy, the polyphosphate "overplus" phenomenon, provides not only *Microcystis*, but other cyanobacteria, with a competitive advantage over many microalgae (Sommer 1985). In a study by Kilham and Hecky (1988), polyphosphate (volutin) granules were synthesized intensely when the cells were transferred after 8 days from medium with a lower P concentration (1.5 mg L^{-1} PO_4-P) to their standard culture medium (10–320 mg L^{-1}P). Polyphosphate synthesis was 25 times higher than in the culture grown for the same time in standard medium. Using flow cytometry it has been shown that GV formation in *M. aeruginosa* is influenced by the P status of the cells. Jacobson and Halmann (1982) showed that a P-starved population produced less GV volume per cell, did not display large variability in the rate of density change of GV and the cell photosynthetic rate was less than in a P-replete culture (Jacobson and Halmann 1982). In spite of the reduction in GV volume in P-limited cultures, there was minimal change in buoyancy, because of an increase in carbohydrate. However, Brookes et al. (2000) found that the greatest buoyancy loss occurred with the highest P-treatment (10 μM).

The start of GV synthesis in a P-limited *Microcystis* culture needed at least 10 h light after a P pulse (Kromkamp et al. 1989a). P-limited *Microcystis* outcompeted *Oscillatoria* due to the lower inhibition constant k (see above) and V_{max} (110 and 30 μg P per mg protein, respectively). With these storage attributes *Microcystis* can form six times more new cells than *Oscillatoria*. *Microcystis* with its adaptation to P limitation by increasing its P uptake capacity and decreasing its light harvesting capacity may be an important organism in waters with a fluctuating P-supply (Brookes and Ganf 2001). This storage strategy combined with the ability to control buoyancy enables *Microcystis* to gain access to P in deeper water layers when epilimnetic P concentrations are low (Ganf and Oliver 1982). Another feature of *Microcystis* relevant to its dominance and persistence in fluctuating P conditions is its ability to exist in different colony sizes. The growth of unicellular and small colonial *Microcystis* strains was significantly inhibited at 0.2 mg L^{-1} P, however, whereas large colonial strains were not inhibited. Moreover the colonial strains had a higher affinity for P at low concentrations, while the unicellular strains consumed more P than the colonial strains. Alkaline phosphatase activity in the unicellular strains was significantly induced by low P concentrations. Under P- limited conditions, the oxygen evolution rate, Fv/Fm and maximum electron transfer rate were lower in unicellular strains than in colonial strains (Shen and Song 2007). Colony-forming *M. aeruginosa* accumulated P better than *Pseudomonas* from P-containing organic sources (Yuan et al. 2009). This ability is very useful, especially if phosphate concentrations in the water are low during bloom periods, but also during overwintering in the benthic phase.

It has been suggested that the P metabolism of *Microcystis aeruginosa* is linked to redox potential (Eh). P release was accelerated in the dark when the redox potential was lowered. A low redox potential value in darkness stimulated the accumulation of polyphosphate and the degradation of polyglucose; the polyphosphate synthesis delayed the decrease of intracellular orthophosphate. Cell mortality was reduced when the redox potential was low in the dark. The accumulation of polyphosphate under low redox potential conditions in the dark was very important for survival under growth conditions otherwise unfavourable for maintaining P concentration, energy storage, and other physiological functions. The ability to accumulate polyphosphate in the dark and negative redox potential values may be of considerable advantage in low-light, organically rich and low-redox potential habitats. Furthermore, Davis et al. (2009) have shown that increases in temperature and P concentration usually yield the highest growth rates for toxic *Microcystis* cells, suggesting that future eutrophication and climatic warming may have an additive effect in promoting the growth of toxic, rather than non-toxic, populations, leading to blooms with higher microcystin content. However, a negative relationship has been reported between microcystins contents and P content (Downing et al. 2005).

Overall, *Microcystis* plays a crucial role in the phosphorus cycle in lakes. Colonies of *Microcystis* assimilate P in the sediment prior to their migration into the pelagial region and use this internal store to support their planktonic growth. Epilimnetic growth based solely on internally stored P can last for 15–20 days under 'optimal' growth conditions (Istvánovics et al. 1993). There was a persistent coincidence between the occurrence of *Microcystis* blooms and an increase in both total P (TP) and soluble reactive P (SRP) concentrations in the water of enclosures with sediments. *Microcystis* blooms induced massive release of P from the sediment, perhaps mediated by the high pH caused by intense photosynthesis and/or depressed concentrations of NO_3-N (Xie et al. 2003b). On the other hand P stored by *Microcystis* spp. can be released during decomposition and represents an important potential source of internal P loading (Wang and Chen 2008).

7.4.5.3 Nitrogen

N is an essential component in the synthesis of gas vesicles**,** and N limitation may have an impact on cell buoyancy. *Microcystis* can acquire N as NO_3, NO_2 or NH_4^+, but not as N_2, because of the absence of nitrogenase. However, as

some genera of non-heterocystous cyanobacteria can fix N_2, although mostly under micro-oxic or anoxic conditions (Dugdale et al. 1961; Wyatt and Silvey 1969; Carpenter and Price 1976; Bergman et al. 1997), the possibility of N_2 fixation occurring in the centre of *Microcystis* colonies or in scums should be checked more thoroughly. In general the order of the preference for cyanobacteria is NH_4-N > NO_3-N > N_2 (Tandeau de Marsac and Houmard 1993) and the impact of NH_4-N on *Microcystis* physiology is ten times more than NO_3-N. In the presence of NH_4-N the growth rate should be 4.6 times higher than for NO_3-N (Sirenko 1972).

N metabolism is strongly connected with carbon fixation. The formation of amino-N from nitrate (requirement 4 e^-) is closely related with assimilation of carbon dioxide to carbohydrate (requirement 10 e^-), so both processes compete for energy and reductant generated by photosynthesis (Oliver and Ganf 2000). Further interactions between N and C occur during their incorporation into protein. N assimilation influences the rate of CO_2 fixation and the level of carbohydrate reserves with a significant impact on cell growth, cell turgor pressure and cell density, and C and N limitation have opposite effects on buoyancy (Klemer et al. 1982). The accumulation of intracellular nitrite could be the cause of inhibition of *Microcystis* growth and photosynthesis under high nitrate concentrations during high CO_2 conditions (Chen et al. 2009b). N-limited *Microcystis* cultures show a reduction in GV formation and an increased in cellular carbohydrate contents, resulting in a loss of buoyancy. When N was not limited (100 μM) increased GV volume, metabolized carbohydrate more efficiently than N-limited culture and retained positive buoyancy (Kromkamp et al. 1989b). When various NH_4-N supply rates were tested in mixed chemostat cultures, *Microcystis* was dominant only at the slowest dilution rate (0.1 day^{-1}), while *Scenedesmus quadricauda* was dominant at the higher (0.3 day^{-1}, 0.8 day^{-1}) rates (Brookes and Ganf 2001). In nature the increased availability of NH_4-N common in the hypolimnion of stratified lakes may cause a reduction in the carbohydrate reserves of cyanobacteria leading to a quicker reversal of cell buoyancy and a reduction in the extent of vertical migration (Oliver and Ganf 2000).

Xu et al. (2010) suggest that N is the primary limiting nutrient for *Microcystis*, with P being a secondarily limiting nutrient. When P enrichment is >0.20 mg L^{-1} and N enrichment >0.80 mg L^{-1}, growth of bloom-forming *Microcystis* is not nutrient limited. The availability of N during the summer is a key factor potentially limiting growth of toxic *Microcystis* blooms. Therefore, although reducing the P load is important, reducing the N load is essential for controlling the magnitude and duration of *Microcystis* blooms.

Cellular microcystin quotas of *M. aeruginosa* showed a significant positive relationship with both nitrate uptake and cellular N content and a negative relationship with CO_2 fixation, P uptake, and cellular P content (Downing et al. 2005). Thus, the ratio of nitrate uptake to phosphate uptake, cellular N to cellular P, and nitrate uptake to CO_2 fixation were positively correlated to cellular microcystin. Nonanoic acid stress had no effect on NO_3-N uptake, but could stimulate P uptake (Shao et al. 2009). An increase in ambient NO_2-N up to 10 mg L^{-1} promoted microcystin (MC-LR) production by *M. aeruginosa* PCC7806; above this concentration the growth was inhibited by oxidative stress, but extracellular MC-LR continued to increase (Chen et al. 2011).

7.4.5.4 N:P Ratio

Of the major hypotheses that have been proposed to explain the success of cyanobacteria, the most prevalent and disputed explanation may be that of the TN:TP ratio ("TN:TP rule"). Smith (1983) analyzed the data set from 17 lakes and concluded that low N to P ratios (<29 by mass) favour dominance of cyanobacteria. The review by Hyenstrand et al. (1998) considered a low N:P ratio (29:1) as one of the nine main factors affecting the success of cyanobacteria. On the other hand, high N:P ratios (20–50:1) lead to a community dominated by green algae (Bulgakov and Levich 1999) or diatoms (McCarthy et al. 2009). The optimal growth rate of *Microcystis aeruginosa* (1.6 divisions day^{-1}) isolated from Lake Biwa occurred when the N:P ratio is 100:1 and declines at lower ratios (N limitation) and higher ratios (P limitation). In contrast, *Anaba*ena sp. growth rates did not change at N:P ratios from 1,000:1 to 10:1 (Nalewajko and Murphy 2001). In nature *Microcystis* was most successful with a mean TN:TP ratio of 70:1 (mol:mol), whereas *Aphanizomenon* thrived with a mean ratio of 48:1 (Lehman et al. 2009). Sabour et al. (2009) concluded that a markedly diminished growth in response to a lower N:P ratio occurred only for *Microcystis* and in the highest N:P ratio tested for *M. ichthyoblabe*, as well as *Anabaena aphanizomenoides*. It has been suggested that the form of N may also play a key role, with higher NO_3-N levels shifting the advantage to *Microcystis* (Lehman et al. 2009). Nevertheless, Moisander et al. (2009) found that *Microcystis* abundance increased in response to all forms of N; when N was not limiting, the doubling time was 1.24–1.39 days. The authors suggested that the availability of N during the summer is a key factor influencing the initiation and maintenance of toxic *Microcystis* blooms.

However, a comparative study on two subtropical lakes suggests that resource-ratio explanations for cyanobacterial dominance may not apply. In Lake Okeechobee, Florida, the cyanobacterial proportion increased with increasing TN:TP, but in Lake Taihu, China, cyanobacteria decreased with increasing TN:TP (McCarthy et al. 2009). These contrasting results may explain the study by Xie et al. (2003a) that *Microcystis* blooms were limited by the available N and P (increasing P concentrations) rather than a decrease in the their ratio. The cure sounds simple: decrease inputs of the limiting nutrient. But which nutrient and how deeply

should inputs be cut, when the human activity has greatly increased? Global flux of reactive N to the biosphere from food production has increased from ≈15 Tg N year^{-1} in 1,860 to ≈187 Tg N year^{-1} in 2005 (Galloway et al. 2008). The global P flux to the biosphere increased from ≈ 10–15 Tg P year^{-1} in preindustrial times to ≈33–39 Tg P year^{-1} in 2000 (Bennett et al. 2001). The results presented by Schindler (1977) from a 37-year experiment on nutrient management in Canadian lakes show that a single-minded focus on control of reactive N would have disastrous consequences for aquatic resources. To decrease eutrophication, the control of reactive N alone is not sufficient. P control is essential and must be included in management programmes designed to decrease eutrophication of freshwaters and coastal zones (Carpenter 2008).

In conclusion, a counter view is advanced to the opinion that the N:P ratio is an independent factor that regulates the assemblage composition in the phytoplankton. The initial ratios of resource availability provide a valuable indication of the capacity of a system to support phytoplankton biomass and suggest the probable outcome of autogenic processes under relatively steady-state conditions with a chronic resource limitation. An alternative view of community assembly suggests that most species of algae will grow under a wide range of environmental conditions. Which phytoplankton species will dominate depends upon the interaction of many complex factors. The ability to maintain growth under conditions of suboptimal nutrient supply may eventually prove to become selectively crucial (Reynolds 1999).

7.4.5.5 Carbon

Carbon dioxide is the main source of inorganic carbon for *Microcystis,* but it can also utilize other forms of carbon. The concentration and speciation of dissolved inorganic carbon is strongly linked to pH through equilibrium reactions between the species CO_2, H_2CO_3, HCO^{3-} and CO_3^{2-}. The proportion of CO_2 declines from a maximum at pH 4 to only 0.003% of the total inorganic C concentration at pH 9. Low CO_2 concentrations can give an advantage to gas-vacuolate cyanobacteria (Shapiro 1990). Mixed batch culture experiments where a species of each of *Microcystis, Staurastrum* and *Synedra* were tested under three pH values (8.2, 8.8, 10.2) resulted in the dominance of *Microcystis* in all cases. The growth of *Microcystis* appears to be related to its advantageous CO_2 uptake and having the lowest half-saturation constant of the three organisms, reflecting its high affinity for dissolved inorganic carbon (Yamamoto and Nakahara 2005).

Paerl (1983) used $^{14}CO_2$ followed by microautoradiographs to show that fixation by unstirred *M. aeruginosa* colonies was initially confined to peripheral cells, but when $^{14}CO_2$ was allowed to diffuse into colonies 15 min before illumination, a more uniform distribution of labeling was observed. Among colonies located at the air-water interface, internal cells showed an increased share of photosynthate production when atmospheric $^{14}CO_2$ was supplied. This indicated that C_i transport was restricted in large colonies below the water surface, forcing internal cells to maintain a high degree of buoyancy, thus promoting the formation of surface scums. At the surface, C_i restrictions were alleviated. Scum formation appears to have an ecological function, allowing the organism access to atmospheric CO_2 when the C_i concentration is growth-limiting in the water column.

Carbon is fixed to glycogen, which is mobilized by enzymic activity which increases in the dark and thus keeping a constant turnover of the glycogen (Takeya et al. 2004). As a consequence, carbon plays a role in buoyancy regulation. Although CO_2 limitation of photosynthesis can promote buoyancy in the short term by preventing the collapse of turgor-sensitive GV and/or by limiting polysaccharide accumulation, sustained carbon limitation restricts buoyancy regulation by limiting GV as well as polysaccharide synthesis. These results may help to explain the pattern of cyanobacterial dominance in P-enriched, low-carbon lakes (positive effects of bicarbonate enrichment on cyanobacterial N uptake) (Klemer et al. 1996). An additional (and perhaps quite standard) source of energy can also be exogenous organic substances in a lake rich in organic matter (Sirenko 1972). During *Microcystis* cell lysis (rate 0.094 day^{-1}) total particulate carbohydrate concentration decreased rapidly. The fluctuation of dissolved organic carbon concentration is a function of the production of non-carbohydrate and the decomposition of carbohydrate by bacteria. Total dissolved carbohydrates and dissolved polysaccharide concentrations showed a similar pattern. In contrast, the concentration of dissolved monosaccharides remained constant (Ye et al. 2010). Further information on carbon and its role in physiological ecology are given in Chap. 17.

7.4.5.6 Iron

Although iron is an essential trace element for phytoplankton, its effects on growth are less well understood. However, many studies indicate that iron may be one of the factors which can influence the development of mass blooms of cyanobacteria. Its bioavailability for phytoplankton depends on its concentration and form in the environment (Nedzi and Kosakowska 2005). A low concentration of Fe^{3+} (0.01 µmol) prevented *Microcystis wesenbergii* growth and chlorophyll synthesis, but induced a temporary increase of ATPase activities. Iron addition resulted in the gradual restoration of enzyme structures and functions. Cells in 10 µmol Fe^{3+} grew normally. Cellular chlorophyll *a* and phycocyanin contents were saturated when the iron concentration was above 12.3 µmol L^{-1} Fe and declined slightly at 24.6 µmol L^{-1} Fe (Wang et al. 2010b). Above 100 µmol Fe^{3+} there were temporary increases in the activities of ACP and ALP enzymes due to the formation ferric phosphate

complexes and consequent lack of bioavailable phosphate. Thus too low and too high iron can both have obvious effects on the physiological and biochemical characteristics of *M. wesenbergii* (Xing et al. 2008).

Dissolved Fe is supplied to the hypolimnion during stratification by diffusion of Fe^{2+} from the sediments into the overlying anoxic water as well as by reduction of iron oxide particles settling through the anoxic water column. The peak of particulate Fe occur in the water column of Lake Sammamish, Washington (USA), occurred from July through November (Balistrieri et al. 1992). A kinetic model incorporating the mechanism of Fe uptake by *M. aeruginosa* with particular attention given to the effect of Fe^{2+} and Fe^{3+} was successfully described by Fujii et al. (2010). The results of the iron uptake experiments suggest that iron uptake rates are independent of the cell quota of iron for *M. aeruginosa* and highly dependent on the cell quota for *Planktothrix agardhii*. A kinetic model was developed and simulation suggested that *M. aeruginosa* is a superior competitor under iron limitation (Nagai et al. 2007; Ou et al. 2006).

The dominance of *Microcystis* is determined also by the K:Fe atomic ratio and this does appear to reflect the extent of cyanobacterial bloom more precisely than K or Fe alone. The K:Fe ratio has been found to correlate positively with cyanobacterial percentage, cell number and phycocyanin concentration. With a K:Fe atomic ratio >200, chlorophyll-a concentration, cyanobacterial cell density, and phycocyanin concentration exceeded 10 μg L^{-1}, 20,000 cells L^{-1}, and 20 pM, respectively, the general criteria for eutrophic water (Ahn et al. 2004). The amount of iron can also affect the carbohydrate content. During prolonged P and Fe depletion in O-2 medium, the carbohydrate content increased from 5–8% to 35–60% dry weight (Bickel et al. 2000). The cellular protein content averaged 65% dry weight when grown in complete O-2 medium and decreased during P and Fe depletion to 35% dry weight.

The Fe concentration can affect not only the growth of *Microcystis*, but also its toxicity (Ginn et al. 2009; Li et al. 2009). Fe-limited conditions influenced toxin production by *M. aeruginosa*, and Fe uptake was light dependent (Utkilen and Gjolme 1995). Fe can have a harmful effect on *Microcystis* growth through complexation with dissolved organic matter (DOM) (Nagai et al. 2006). At Fe concentrations <2.5 μM the cells grew much more slowly, but produced 20–40% more toxin. This is in agreement with the hypothesis that production of microcystins may be a response to specific environmental stress conditions (Lukac and Aegerter 1993). The critical values that may stimulate the rapid growth of *Microcystis* and microcystin production were determined to be >0.01 mmol Fe (Jiang et al. 2008). Moreover only MC-LR (no RR) was detected within *M. aeruginosa* cells in cultures with adequate Fe (Yan et al. 2004). Promoter regions of the mcy operon from *M. aeruginosa*, which is responsible for microcystin synthesis, exhibit sequences similar to those sequences recognized by ferric uptake regulator (FUR). This DNA-binding protein is a sensor of iron availability and oxidative stress. In the presence of Fe^{2+}, a dimer of FUR binds the iron-boxes in their target genes, repressing their expression. When iron is absent, the expression of those gene products is allowed. FUR from *M. aeruginosa* binds *in vitro* promoter regions of several mcy genes, which suggests that FUR might regulate microcystin synthesis. The binding affinity is increased by the presence of metal and DTT, suggesting a response to Fe availability and redox status of the cell (Martin-Luna et al. 2006a, b).

7.4.6 Bioturbance and Methane Bubbles

During the reinvasion of colonies from sediments, biotic factors play an important role as well abiotic factors (Bostrom et al. 1989). Successfully overwintering colonies can be deposited in the surface layers of sediment at a depth of usually 2–10 cm and further success of the population requires these to be transported from the sediment surface. Bioperturbation plays an important role in this process, moving the sediments and releasing colonies from the sediments. The most commonly mentioned sources of bioperturbation are methane bubbles produced by bacteria (can release colonies from 10 to 15 cm), zoobenthos (release colonies from 2 to 16 cm) and benthofagous fish (5–20 cm, heavy carp up to 25 cm, depending on sediment composition).

Methanogenesis in sediments increases with depth and depends on the season, although activity can be important even in winter. Strayer and Tiedje (1978) found that the amount of methane increased from January to a maximum in August (35 mmol m^{-2} day^{-1}), and then declines. Methane production is higher in sediments with high amount of organic compounds, but low sulphate (Boudreau et al. 2001). Laboratory experiments by the present authors in transparent cylinders (diameter 25 cm, height 1 m) filled with 50 cm of sediments with *Microcystis* colonies at particular positions and covered by sediments without *Microcystis* showed that *Microcystis* colonies can be released by gas bubbles even from 25 cm depth within a few days. The rate depends on the bubble intensity and composition (structure) of the sediments.

A direct effect of *Chironomus* larvae has been recorded on migration of *Microcystis* colonies from sediment depths of 6–12 cm (Fukuhara 1987). Backward transport of these colonies with the same family of larvae on the sediment surface was also observed (Stahl-Delbanco and Hansson 2002). These larvae produced in the sediment tubes up to a depth of 15 cm (Granéli 1979). The transport of *Microcystis* colonies from the lower layers of sediment to the surface also contributes to their attraction for zoobenthos and fish, which contribute to sediment movement in natural conditions.

Understanding the influence of bioturbation on physical, chemical and biological processes of the water-sediment interface requires investigating top-down (consumer) and bottom-up (resource) forces.

The interaction between sediment properties, the physiological state of *Microcystis* colonies and the influence of bacteria, zoobenthos and fish in causing bioturbation are usually the main parameters considered in the study of *Microcystis* recruitment from sediments. A conceptual model that highlights the importance of sediment structure on bioturbation and the evidence for the integration of bottom-up influence on zoobenthos activities was suggested by Nogaro et al. (2009). However, macro-invertebrates and aquatic plants often play key roles in biogeochemical processes at the water-sediment interface, but only a few studies have investigated their influence on ecological processes in freshwater sediments, such as nutrient flux, benthic oxygen uptake and microbial activity. The effects of the invertebrate *Tubifex tubifex*, a submerged plant with high sediment-oxygenating potential (*Myriophyllum spicatum*) and a submerged plant with low sediment-oxygenating potential (*Elodea canadensis*) were compared by Mermillod-Blondin et al. (2008). The tubificid worms significantly increased N fluxes of at the water-sediment interface (influx of nitrate to sediment, efflux of ammonium), whereas the two plant species did not have significant effect on these N fluxes. The different effect of tubificid worms was probably due to the bioirrigation process caused by *T. tubifex*, which increased water exchanges at the water-sediment interface. *T. tubifex* and *Myriophyllum spicatum* produced comparable reductions in nutrient concentrations of pore water and comparable stimulations of benthic oxygen uptake and microbial activity judged by percentage active eubacteria and hydrolytic activity, whereas *E. canadensis* had a very weak influence on these variables (Mermillod-Blondin et al. 2008). The role of macrophytes on the water mixing has received little study, but we can, for example, observe significantly lower water transparency in the shallow parts of ponds without submerged vegetation, when they are influenced by strong winds, in contrast to parts with macrophytes.

The influence of macrozoobenthos on *in situ* pore water phosphate concentrations was investigated by Lewandowski et al. (2005). Two-dimensional pore water samplers with a high spatial resolution were exposed for 14 days at two sampling points at different water depths. Macrozoobenthos densities and the corresponding pore water phosphate concentrations were determined. It was found that macrozoobenthos affected the sediment environment mainly through bioirrigation, bioturbation, secretion and digestion. It is most likely that there are hot spots caused by secretions from chironomids which intensify the microbially mediated P-release. Chironomid larvae can further accelerate nutrient enrichment in a eutrophic system that may encourage a "snow ball effect" towards a hypereutrophic one. The counts of both heterotrophic and phosphate solubilizing bacteria show strong positive correlation with orthophosphate concentration in water and the correlation also exists between organic carbon concentration in sediment and phosphate in overlying water. This implies that the accelerated phosphate flux was the result of coordinated eco-engineering activities of chironomid larvae and microbe-mediated mineralization of organic matter (Biswas et al. 2009).

The influence on nitrification and denitrification due to bioturbation by the oligochaetes *Limnodrilus* sp. and *Tubifex tubifex* was investigated in eutrophic lake sediment from the Basin of Lake Ringsjon in southern Sweden by Svensson et al. (2001). Denitrification was stimulated by the oligochaetes more at high concentrations of nitrate in the overlying water than at low concentrations. Comparison of the enhancement of denitrification by oligochaetes with other similar studies of denitrification in eutrophic sediment bioturbated by tube-dwelling chironomids indicates that a similar biomass oligochaetes is less effective at mobilizing nitrate to deeper sediment layers. Sieving and homogenising the sediment had little effect on the rates of denitrification and nitrification compared to undisturbed sediment. Lagauzere et al. (2009) investigated the influence of *Chironomus ripanius* and *Tubifex tubifex* on another process, diffusive oxygen uptake (DOU). The two species had significantly increased the DOU of sediments significantly (13–14%) by 72 h, but there no further changes subsequently. These various findings indicate the importance of studying the effects of macroinvertebrates on sediments when considering the physiology of overwintering *Microcystis* colonies, because of the influence the animals can have on microbial activity and nutrient availability. Factors affecting macroinvertebrates such as pollutants may therefore also affect overwintering *Microcystis*.

A study of bioturbation by the red swamp crayfish *Procambarus clarkii* in a pond looked at its influence on cyanobacteria at various times of year (Yamamoto 2010). The presence of the crayfish in containers of pond sediment increased the densities of cyanobacteria such as *Microcystis* spp. and *Anabaena* spp., while their population densities began to decline when the crayfish was removed. It was concluded that bioturbation by crayfish is important in the dynamics of blooms in the pond, since their effect varied with sex and season. Males may play an active role in the initiation of the bloom in late spring, while females may contribute to extension of the bloom in late autumn. Both males and females contribute equally to the maintenance of the bloom from summer to autumn.

Although little is known about degradation of *Microcystis* in sediments, studies have been made on its breakdown by bacterial communities during anaerobic digestion. For instance, Zeng et al. (2010) quantified the effect of changes in the ratio of bacterial community to *Microcystis* on methane formation and orthophosphate release.

While there is still much to be investigated on the influence of bioperturbation on *Microcystis*, it seems clear that any activity which enhances the oxygen concentration in the upper layer of sediments if likely to be unfavourable for *Microcystis* resting colonies. In contrast microbial bioperturbation by bubble mixing establishes more positive conditions for *Microcystis* e.g. low oxygen and high P concentrations.

7.5 Interactions with Other Organisms

Some of the diverse ways Microcystis interacts with other organisms have already been mentioned, but two need to be considered in more detail – zooplankton grazers and bacteria.

7.5.1 Grazing by Zooplankton

7.5.1.1 Mechanical Interference

Grazing is one of the major factors modifying the phytoplankton biomass and the four main zooplankton groups involved are rhizopods, ciliates, rotifers and crustaceans (mainly cladoceran and copepods), though a number of other groups can also be important, such as some mixotrophs. The zooplankton have diverse means of selecting, obtaining and ingesting food organisms (Reynolds 2006) and size is one factor. However, it is not always easy to establish whether the responses of *Microcystis* and other planktonic cyanobacteria to grazers are entirely mechanical or also involve chemical interactions. The uptake of filamentous and colonial cyanobacteria by filter-feeding zooplankton has been a matter of considerable discussion (Gulati et al. 2001; Paterson et al. 2002), but the response does seem to be largely, if not entirely, mechanical in some cases. For instance Lampert (1987) concluded that larger *Microcystis* colonies have in general a depressive effect on filter feeding due to mechanical interference with the feeding apparatus. However, doubt about even this is raised by Visser et al. (2005), who commented that size alone seems to an unlikely criterion for rejection by a grazer. Ger et al. (2010) found large differences in the ingestion of *Microcystis* cells by two calenoid copepods, but no difference during a short feeding experiment according to whether the strains were microcystin producing (MC+) or lacking (MC–). *Ochromonas* sp., a mixotrophic flagellate, was also able to feed on *Microcystis aeruginosa* strains of varying toxicity (Van Donk et al. 2009). It is unclear how large a colony can be attacked by a particular size of *Ochromonas* cell, but the literature has examples of *Ochromonas* engulfing cells considerably larger than itself. Colony density can also have an effect, since Chen et al. (2007) found that reducing the density of *Microcystis* colonies led to a shift in dominance by small-sized cladocerans to dominance by large-sized daphnids.

Interpretation of past studies on *M. aeruginosa* is complicated by the fact that the presence of grazers can induce colony formation, making it important to differentiate between short- and long-term responses to grazing. Colony formation can be considered an inducible defence against flagellate grazing under conditions where toxins cannot deter such grazing effectively (Yang et al. 2006). In a subsequent paper, the same authors (Yang et al. 2008) described how grazing enhanced colony formation and the synthesis and secretion of extracellular polysaccharide by *M. aeruginosa*. However, the opposite effect can occur with other grazers. *M. aeruginosa* populations grazed by the copepod *Eudiaptomus graciloides*, the cladoceran *Daphnia magna* and the rotifer *Brachionus calyciflorus* were all strongly dominated by unicells and few or no colonies occurred (Becker 2010). Observations (Boing et al. 1998) in Bautzen Reservoir, Germany, suggested that *Microcystis* is the main planktonic phototroph resistant to grazing there, and it seems probable that this applies to many other waterbodies. When grazing was absent, strong competitive interactions occurred between *Microcystis* strains, whereas indirect positive interactions were prevalent in the presence of a generalist grazer (Van Gremberghe et al. 2009b). It is clear that understanding the effects of grazers requires chemical interactions to be considered as well as mechanical effects.

7.5.1.2 Chemical Warfare

Until relatively recently the functioning of ecosystems was considered mainly with respect to the flows of biomass and energy between trophic levels. However, it is becoming increasingly clear that there is exchange of information between these levels facilitated by the release of infochemicals by the organisms. The influence of some grazers on colony formation by *Microcystis aeruginosa* described above probably provides an example, although any chemical compounds involved have apparently not so far been characterized. Chemical communication among freshwater organisms mediates many aspects of predation and interspecific competition and has key roles in determining community structure and ecosystem functioning (Van Donk 2006). Van Donk (2007) proposed that integrating the available knowledge about the role of chemical communication into models should be one of the aims of ecological informatics. It is therefore important to consider in more detail how infochemicals may affect the interaction between zooplankton and *Microcystis* within planktonic food webs.

Microcystins are the most studied potential infochemicals produced by *Microcystis* as response to grazing. Inhibition of zooplankton grazing has been shown to occur in response to microcystins (Fulton and Paerl 1987; Lampert 1981, 1982). It has also been shown that *Microcystis* strains exposed to

zooplankton increased their cell specific toxin production (Jang et al. 2003). Several lines of evidence suggest that microcystins may have an allelopathic role, since they act as potent inhibitors of protein phosphatases 1 and 2A in both plants and animals (MacKintosh et al. 1990). Another important mechanism whereby microcystins can have an impact seems to be oxidative stress: excess formation of reactive oxygen species (ROS) might cause serious cellular damage including peroxidation of lipid membranes, genotoxicity and induction of apoptosis.

As only a limited number of laboratory studies have described harmful effects of microcystins at concentrations typically found in nature, Babica et al. (2006) cautioned that the role of microcystins on phototrophs should not be considered simply as one of toxicity. Nevertheless a lot of research has been carried out showing their toxic effects on various zooplankton species (Wiegand and Pflugmacher 2005). A study of six lakes led Hansson et al. (2007) to conclude that microcystins were playing an important role in chemical warfare against herbivores. The concentrations of microcystins generally showed a bimodal pattern, with peaks in early summer and in autumn, and total zooplankton biomass was negatively correlated with microcystin concentration. Separating the zooplankton assemblages into finer taxonomic groups revealed that high microcystins concentrations were negatively correlated with *Daphnia* and calanoid copepods, but positively correlated with small, relatively inefficient phytoplankton feeders, such as cyclopoid copepods, *Bosmina* and rotifers. Further, changes of microcystins were coupled with reduced adult size and diminished juvenile biomass in *Daphnia*. Consequently, large unselective herbivores, such as *Daphnia*, are 'sandwiched' between high fish predation and toxic food (cyanobacteria). Thus, susceptibility to *Microcystis* and its toxins seems to depend on the zooplankton species (Wiegand and Pflugmacher 2005). In the similar way high population densities of naked amoebae grazing on *Microcystis* coincided with rapid decreases in *Microcystis* biomass simultaneously with the change its genetic structure – *M. aeruginosa* was grazing more than *M. viridis* (Van Wichelen et al. 2010).

A study of the hypertrophic Villerest Reservoir in France also showed that *M. aeruginosa* can affect grazers in different ways (Aleya et al. 2006). *Daphnia*, which was included in this study, has probably been investigated more than other genus of grazers interacting with *Microcystis*. However, it is still difficult to make firm generalizations. In the case of the Villerest Reservoir, *Daphnia longispina* did not seem to suffer from the proliferation of the *M. aeruginosa*; in fact its biomass decreased as the bloom decreased. Two other grazers showed different responses. *Cyclops vicinus* seemed to move away when *Microcystis* invaded the superficial layers of the reservoir. Its ability to migrate permitted it to move to the littoral zone, where food was available. Another copepod, *Eudiaptomus gracilis*, underwent diapause as *M. aeruginosa* increased. In contrast with the observation of Aleya et al. (2006) for *Daphnia longispina*, Deng et al. (2010) commented on the frequently observed midsummer decline of *Daphnia* in *Microcystis*-dominated lakes. Their laboratory study on *Daphnia carinata* and *D. pulex* showed decreases in both in response to increases in *Microcystis aeruginosa*, with the decrease being more marked in *D. carinata*, which is the larger species. The authors ascribed the decreases to mechanical interference by *Microcystis* colonies. The presence of *Microcystis* in the diet led to marked differences in formation of ephippia by the two cladocera, with a positive effect in *D. pulex* and a negative one in *D. carinata*. However, the percentage of empty ephippia (i.e. no resting eggs) was higher in both. Although the authors did not mention it, this raises the possibility that toxins may have played a role in this study as well as mechanical effects.

The influence of grazers on microcystin content reported by Jang et al. (2003) mentioned earlier was investigated in much more detail by Jang et al. (2007) in a laboratory study on the influence of *D. magna* and *Moina macrocarpa* on three *Microcystis aeruginosa* strains and one *Planktothrix agardhii*. Microcystin production increased after exposure to both grazers and also filtrates of the medium in which the animals had been grown – termed infochemicals by the authors. The higher the grazer density or the higher the infochemical concentration, the more microcystin was formed, this effect being significant in most cases. Cyanobacteria directly exposed to *Daphnia* released greater amounts of microcystins (i.e. extracellular) than those exposed to *Moina*. Another study (Becker 2010) with *D. magna* and *Microcystis aeruginosa*, in this case a toxic and a non-toxic strain, showed that *Daphnia* ingested more non-toxic than toxic cells, and survived longer with non-toxic cells. Addition of cell-free extract from disrupted toxic *Microcystis* cells strongly increased the aggregation of the intact cells with growth impaired by being grown under low light (and perhaps also the presence of bacteria). *Chlamydomonas* was the food source for growing the *Daphnia* used in this experiment and the author pointed out the need to investigate the effect of food source on the interactions between *Microcystis* and *Daphnia*.

The influence of other phototrophs has also been investigated in studies where *Daphnia* has access to these phototrophs as well as *Microcystis*. The sequence in which populations of other phototrophs develop among *Microcystis* populations can have a lasting influence on community and population structure, a phenomenon referred to a priority effect (Van Gremberghe et al. 2009a). Laboratory studies indicate that priority effects may have a profound impact on the strain composition of *Microcystis* populations. When *Chlorella vulgaris* or *Scenedesmus acutus* where present as well, *Microcystis aeruginosa* supported the population increase of

Daphnia pulex better than when offered alone (Alva-Martinez et al. 2004). It is unclear how much these effects are due to the influence of different phototrophs on *Daphnia* or whether interactions between the various phototrophs also play a role. Direct predation on *Microcystis aeruginosa* single-cells has been described by phototrophic flagellate *Ochromonas* sp. (Wang et al. 2010d). These authors demonstrated combined effect of flagellate grazing with nitrogen low concentration on colony formation by *Microcystis aeruginosa.*

The ability of *D. magna* to cope successfully with toxic *Microcystis* was improved when the animals had previously been exposed to toxic *Microcystis* (Gustafsson and Hansson 2004). The authors suggested that the toxin may have less effect on *D. magna* populations that are repeatedly exposed to toxic cyanobacteria in their natural habitat than populations lacking prior exposure. Sarnelle and Wilson (2005) made the rather similar conclusion for *D. pulicaria* populations that, when it was exposed to high cyanobacterial levels over long periods of time, it can adapt to being more tolerant of toxic cyanobacteria. A growth study (Sarnelle et al. 2010) involving a comparison of a toxic and a non-toxic strain of *D. magna* showed that there was no effect of toxin presence on per-capita fecundity of surviving adults, the animal produced smaller neonates when fed toxin-containing *M. aeruginosa* than when fed the non-toxic mutant. Hence, although *Daphnia* survival, population growth and neonate size were negatively affected by microcystin presence, populations with prior experience of toxic cyanobacteria may show positive population growth even at high concentrations of cyanobacterial toxins. An increased tolerance by *D. magna* to microcystin is not only inducible during an individual's lifetime, but can be transferred from mother to offspring (Gustafsson et al. 2005). This maternal effect was expressed in several fitness parameters, including shorter time to maturity and first reproduction, and higher numbers of offspring compared to inexperienced individuals. In some circumstances, such maternal effects could increase population production by up to 40%.

Although the effects of microcystins on zooplankton grazers have been studied intensively, it can still be difficult to make broad generalizations on the effects of grazers on *Microcystis.*. However, Ghadouani et al. (2003) concluded that zooplankton communities do seem to be affected negatively by cyanobacterial blooms and therefore the potential to use herbivory to reduce blooms in eutrophic lakes appears limited. Nevertheless, Wang et al. (2010c) showed that the presence of zooplankton can actually favour bloom formation. Their study investigated the combined effects of phytoplankton community competitive interactions, nutrient enrichment and zooplankton on *Microcystis* bloom formation in water taken from a pond with a very low concentration of *Microcystis*, but high phytoplankton biomass expressed as chlorophyll *a*. The experiment was conducted on temperature and light conditions favourable for *Microcystis* and addition of N and P did promote a surface *Microcystis* bloom. However, when the zooplankton was removed from the water at the start of the experiment, no surface *Microcystis* bloom formed, regardless of nutrient addition. Chlorophyta dominated in the absence of zooplankton, when the same nutrient addition was made. *Microcystis* bloom formation in this eutrophic water body at a mean temperature of about 36°C at 14:00 h was closely related to the initial presence of zooplankton and a sufficient supply of N and P. The authors suggested this was one of the first demonstrations of zooplankton controlling *Microcystis* bloom formation in a waterbody previously free of surface cyanobacterial blooms, though in this case it was the removal of zooplankton rather than their addition which had this effect. The nutrient regime can also affect the relative survival of *Daphnia* strains differing in their microcystin tolerance (Chislock et al. 2011). In an 8-week limnocorral experiment, all six genotypes were equally represented under low N:P (4:1, by atoms), whereas *Daphnia* the most tolerant to *Microcystis* dominated under high N:P (28:1).

When considering microcystins, it is well to remember several other points. The possible role of different toxins has usually been overlooked, yet many studies are not critical enough to be sure that all the observations reported are associated with microcystins. The protease inhibitor microviridin J was found to cause a lethal disruption to the moulting behaviour of *Daphnia* feeding on *Microcystis* (Rohrlack et al. 2004). Semyalo et al. (2009) suggested that *Microcystis* contains substances other than microcystins that reduce the growth and survival of the *Daphnia lumholtzi*. Phylogenetic analysis indicates that microcystin synthetase predated the metazoan lineage, thus dismissing the possibility that microcystins emerged as a means of defence against grazing (Schatz et al. 2007). The original reason for their formation and any possible biological role is not clear. The same applies probably applies to other toxins.

Although the interactions between zooplankton and *Microcystis* are far from fully understood, we suggest that the following conclusions do seem proven:

- Removal of green algae and diatoms by zooplankton grazing favours *Microcystis*
- Production of toxic compounds by *Microcystis* can affect filtering rate and reproductive activity of zooplankton
- Production of info-metabolites can results in colony formation in *Microcystis* and modulated behaviour (migration) of zooplankton.

There is a need for more comprehensive approach, which brings together studies on the interrelationships between zooplankton and colony formation, population dynamics and the annual cycle – the next subject to consider here.

7.5.2 Grazing by Fish

Microcystis can be consumed by herbivorous fish, but, in at least some cases, live colonies can pass through the fish gut, such as in the study on silver carp by Gavel et al. (2004), when *Microcystis* showed high viability. Moreover, live colonies can use directly P available in the fish gut (Lewin et al. 2003). However, phytoplankon-consuming fish can have a marked adverse effect on *Microcystis* populations and Zhang et al. (2008b) have reviewed the potential for using this approach to control nuisance blooms in tropical lakes that are highly productive and where it is almost impossible to reduce nutrient concentrations to sufficiently low levels to have an impact. Their studies showed that silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*) (two filter-feeding planktivorous species commonly used in management) could suppress *Microcystis* blooms efficiently during long-term observations on Lake Donghu and Lake Qiandaohu, China. However, this type of approach was only suitable where there are dense populations of large algal species (such as *Microcystis* colonies). It did not work efficiently in less eutrophic systems where nanophytoplankton dominated. In view of the impact of zooplankton grazers on some *Microcystis* populations (Sect. 7.5.1), it is important to establish role of different types of grazers on different types of population and different morphospecies.

7.5.3 Interactions with Bacteria

Several authors have considered the roles of bacteria in *Microcystis* colonies. Worm and Sondergaard (1998) considered this small ecosystem may be considered even as a bacterial 'incubator' for the surrounding water. Bacteria associated with *M. aeruginosa* used colonies not only as a substrate for colonization, but also as an energy source (Shi et al. 2003). All the bacterial isolates made by these authors grew in a medium in which the only carbon source was *M. aeruginosa* culture filtrate. In a further study, Shi et al. (2010) found that the bacterial communities of the same *Microcystis* morphospecies tended to be similar and associations may be species-specific. Phylotypes related to *Pseudomonas*, *Serratia* and Flexibacteraceae were dominant in colonies of *M. wesenbergii, M. flos-aquae* and *M. aeruginosa*, respectively. Evidence was found by Coelho-Souza et al. (2005) for sulphate-reducing activity, but not mercury methylation, by the bacterial community in *M. aeruginosa* colonies in the hypertrophic Jacarepaguá lagoon in Brazil.

Fig. 7.4 Plankton population on Brno reservoir during August 2006: (**a**) Macroscopic colonies of *Microcystis* sampled from water *top layer* (0–30 cm) by Friedinger (**b**) Biomass of *Microcystis* for toxicological analyses sampled by plankton net (Photo by L. Šejnohová and M. Sadílková)

7.6 Annual Cycle

7.6.1 Plankton Phase – The Water Bloom

Microcystis is known best from the surface and the water column in summer (Fig. 7.4). However, the annual cycle of cells in temperate regions includes overwintering in the upper layers of sediment, reinvasion into the water column in the spring, formation of bloom and sinking of the population in the autumn. The planktonic period in colder temperate regions is sometimes not much more than one-third of the cycle, the remainder occurring in bottom sediments under very different conditions to those in the water. The two populations have different ecological requirements, with differences in morphology (Fig. 7.5, Table 7.2) and type of nutrition. The planktonic photosynthetic population is renewed annually from the benthic population, whereas the benthic heterotrophic population is a permanent stock of colonies that must be re-supplemented by autumn sedimentation. The transitions between populations in spring and autumn involve changes in ecophysiological demands, metabolism and ultrastructure. The life cycle of *Microcystis* has been described most thoroughly for three (morpho-) species. In the case of *M. aeruginosa* from temperate regions (Table 7.2), seven stages have been recognized (Table 7.3) (Strayer and Tiedje 1978; Reynolds et al. 1981). Five stages were reported for the tropical *M. panniformis*, but no significant morphological changes in another tropical

Table 7.2 Phases of annual cycle by *Microcystis* and their affiliation to individual populations, based on Reynolds et al. (1981)

Population	Phase of annual cycle	Morphological status
Plankton	Growth	Ib
	Blooms	Va, VI
	Regulation of buoyancy	II + III
	Collapse of gas vesicles, accumulation of storage products	II + III
	Sinking out	II + III
Benthic	Overwintering	Ia, II + III, IV, Vb
	Reinvasion	IV, Vb

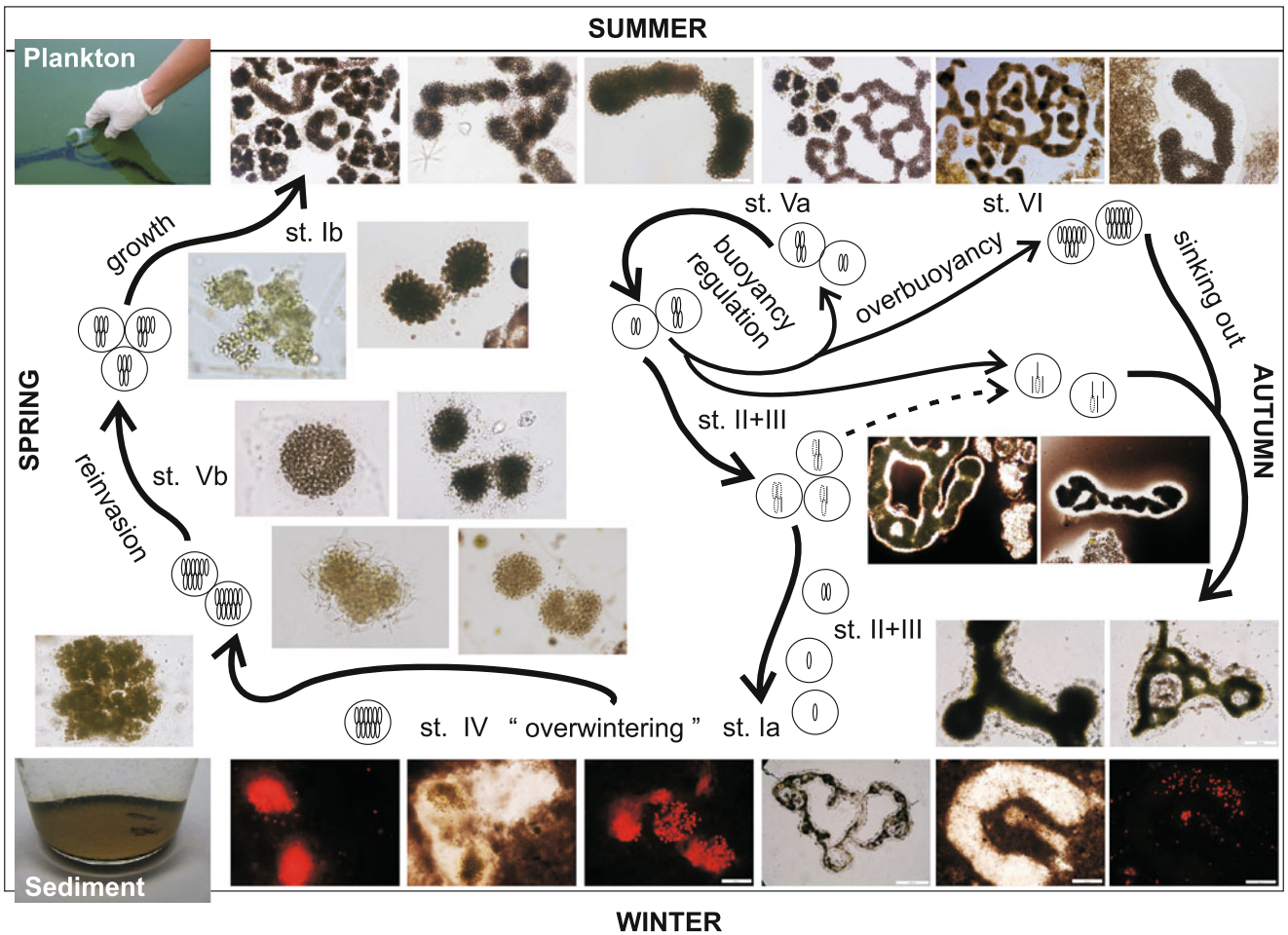

Fig. 7.5 Annual cycle of *Microcystis* in a temperate region lake, according to Reynolds et al. (1981, p. 57) (See Table 7.2 for more details)

Table 7.3 Morphological states of *Microcystis*, based on Reynolds et al. (1981)

Status	Form of colony	Colony size (μm)	Cell arrangement	Mucilage size	Cell density	Physiological state	Population
Ia	Ellipsoid (cultivation invokes st. IV)	40–1,000	Diffuse, cells do not overlap	Large with the numbers of bacteria	<1/1,000 μm^3	Senescent or moribund, empty cells	Benthic (high bacterial activity)
Ib	Irregular oval or cylindrical colonies	Max. 250	As st. Ia	No record	<1/1,000 μm^3 (20–200 living cells/col.)	Without senescent or moribund cells	Early pelagic – exponential growth
II III	Irregular ellipsoid, fenestrated (II) strap-like (III)	40–300	Cells compactly arranged	10–45 μm	>3/1,000 μm^3	Active (cultivation invokes st. Va)	Late pelagic, benthic
IV	Similar description as st. Ia	Not stated	Diffuse with random cluster soft living cells	No record	20–200 living cells/cluster	Status for overwintering or reinvasion	Reinvaded benthic
Va	Oval or quasi-spherical form	80–300 (570)	Relatively compact	3 μm (growth) 35 μm (end of year)	3–5/1,000 μm^3	Active	Pelagic (major part of bloom)
Vb	Small, spherical	40	No record	2–5 μm	<100 cells/col.	Re-establishment of planktonic population	Spring, early pelagic
VI	Lobe formation	No record	No record	No record	No record	Active	Pelagic

species, *M. protocystis* making it quite distinct from the tropical *M. protocystis* sampled from several reservoirs in Brazil and also studied in culture, and from the cosmopolitan and widespread *M. aeruginosa* and *M. flos-aquae* (Komárek et al. 2002).

Some of the differences between planktonic and benthic populations have already been described in Sect. 7.4. Early planktonic colonies (st. Ib) at the beginning of the season are initially small (maximum linear dimension <250 μm and contain 20–200 live cells). When the bloom forms it consists of the summer stages (Fig. 7.5, Table 7.3). The colonies are at first characterized by the relatively compact and even arrangement of the cells within the mucilage, save for a clear peripheral layer (st. Va). The main part of bloom-forming colonies consists of actively growing cells (st. VI) with a high frequency of division. Maximum photosynthetic activity is associated with the period of rapid growth and frequent cell division. Optimum illumination for maximum photosynthetic activity (Pmax) at 25°C during the incubation of strains of three species, *M. aeruginosa*, *M. viridis* and *M. wesenbergii*, was recorded at 240, 240 and 60 μmol photon m^{-2} s^{-1}, respectively (Yagi et al. 1994).

The process of colony formation is important point in understanding the *Microcystis* life-cycle. In a laboratory study of a *M. aeruginosa* strain isolated from Fetsui Reservoir, Taiwan, and grown with stirring in high N and P medium to avoid nutrient limitation, Yamamoto and Shiah (2010) showed that the internal cells tended to grow faster than peripheral cells. They suggested this was due to the light conditions being favourable towards the centre of the colony because of the light reduction by peripheral cells. The high photosynthetic activity of natural populations, such as that observed by Paerl et al. (1985) may be because of this protection provided by peripheral cells. As the velocity of upward migration is lower in small colonies than large ones (Kromkamp and Walsby 1990), the effect of the shift from turbulent to lentic conditions which initiates the formation of an obvious surface bloom is to favour the accumulation of large colonies at the surface and a layer of small ones beneath. Despite the higher risk of photoinhibition of small than large colonies (Ibelings and Mur 1992), the surface scum attenuates radiation reaching the layer of smaller colonies, perhaps improving conditions for their photosynthesis and growth. The scum, is, however, in a better position for CO_2 uptake (Sect. 7.4.5.5). More detailed studies of the differences in behaviour near the surface are needed.

The dynamics of different *Microcystis* species during summer can be understood by the frequency of their dividing cells (Yamamoto and Tsukada 2009). In *M. aeruginosa* and *M. wesenbergii* this increased by day and fell at night during July and August, but there was no such regular pattern in September or October. The *in situ* specific growth rates were estimated from the frequency of dividing cells with time. The values were similar for all three species studied: 0.15–0.38 day^{-1} for *M. aeruginosa*, 0.14–0.63 day^{-1} for *M. viridis*, 0.18–0.61 day^{-1} for *M. wesenbergii*. The specific growth rates in July and August slightly exceeded those in September and October. Analysis of the data for *in situ* specific growth rates suggested that either recruitment from the benthic population or morphological change, rather than massive growth, was at least partly responsible for the observed population changes.

The rate at which population changes occur is of course influenced not only by turbulence, light and CO_2, but also nutrients. Nutrient regeneration depends on the effects of phage, parasitic bacteria and grazers, as described earlier, but also breakdown of unhealthy material, such as surface scums suffering photo-oxidation or material deposited on the shore by winds. A study (Chen et al. 2010) of the potential impact of *Microcystis* blooms on microbial eukaryotic community composition assessed by molecular techniques showed that there were marked shifts during *Microcystis* decomposition on the community, with fungi becoming the dominant organisms. However, apparently no study has yet been conducted to assess the relative importance of prokaryotes and eukaryotes in the breakdown of scums, nor quantitative studies on the roles of particular organisms. It is proved by Romo et al. (2012), that the longer water residence time in dry years and dry seasons increased total cyanobacteria biomass, Microcystis aeruginosa populations and MCYST concentrations in the lake water and seston. Droughts increased water retention time by about 45%, and M. aeruginosa populations and MCYST were 1–2 orders of magnitude higher. Those information are important for the uderstanding of the blooms dymnamics and for the consideration concerning the blooms prevention.

Population turnover during the season, indicated by changes in allele composition at both the ITS and cpcBA loci, was studied by Bozarth et al. (2010). Different ITS and cpcBA genotypes appeared to be dominant at the two periods with peak populations, mid-July and September. Toxicity (microcystin content per cell) and toxigenic potential (mcyB copy number) were lower during the second peak, and the mcyB copy number fell further as the bloom declined. Kim et al. (2010) showed similar results for mcyJ genotypes and the genus-specific cpcBA gene. The ratio of cells with mcyJ genotypes to the total *Microcystis* population in a reservoir was highest (68.3%) at the time of the second population peak, and the microcystin concentration in the water began to increase. A denaturing gradient gel electrophoresis profile analysis of the mcyJ genotypes to monitor changes in the toxic *Microcystis* population showed the appearance of new genotypes and the disappearance of existing genotypes during summer, when compared with samples collected in spring and autumn. Recent study in mezocosmos proved possitive correlation of biomass concentration and microcystis content. In the low- and high-cell addition mesocosms, the initial addition of *Microcystis* sp. cells doubled the starting cell abundance from 500 000 to 1 000 000 cells mL^{-1}, but there was no detectable effect on microcystin quotas. Two further cell additions were made to the high-cell addition mesocosms after 60 and 120 min, increasing densities to 2 900 000 and 7 000 000 cells mL^{-1}, respectively. Both additions resulted in marked increases in microcystin quotas from 0.1 pg $cell^{-1}$ to 0.60 and 1.38 pg $cell^{-1}$, respectively, over the 240 min period (Wood et al. 2012).

A general account of the modelling of cyanobacterial blooms is given in Chap. 6, so only a few comments on *Microcystis* are mentioned here. Visser et al. (1997) were the first to construct a model to simulate migration of *Microcystis* in a quiescent water column and then validate the model with the use of cultures. This *Microcystis*-model has been used in subsequent publications with further modifications (Wallace and Hamilton 1999; Oliver and Ganf 2000; Rabouille et al. 2005). Modelling has focussed on three mechanisms by which GV in *Microcystis* (and other cyanobacteria) can influence buoyancy. These are:

1. Changes in cell density through alterations in cellular composition.
2. Changes in GV content due to increases in turgor pressure resulting from photosynthetic activity: when turgor pressure rises with an increase of irradiance, GV can collapse (Walsby 1980).
3. Changes in GV content by synthesis or their dilution during cell growth with reduced GV synthesis.

Improvement of models will require data from other features of *Microcystis* biology, especially the need to consider the population throughout the annual cycle.

7.6.2 Sinking

Sinking of the late planktonic population to the bottom provides a new source for the benthic population (Fig. 7.6). The very compact late summer colonies with their thick layer of peripheral mucilage have enough storage substances for long-term survival in the sediment. The peripheral layer (typically 10–45 μm) appears to provide effective protection against adverse conditions (Fallon and Brock 1981), including bacterial degradation. According to the description of the life cycle by Reynolds et al. (1981) sedimentation involves a shift from stage V to II + III. The low temperature (<10°C) leads to decrease in the rate of photosynthesis and reduction in the formation of gas vesicles (Oliver 1994). Accumulation of hydrocarbons cause a gradual loss of buoyancy from 90% to 35% and a drop to the bottom of the water column (Oliver and Walsby 1984; Thomas and Walsby 1986; Visser et al. 1995). Phosphorus is accumulated in polyphosphate granules during the autumn, which are used as a source of energy during hibernation and re-invasion into the water column (Kromkamp 1987). The influence of colony size on vertical colony migration was included in the modelling study of Visser et al. (1997), who confirmed the suggestion of Reynolds and Walsby (1975) that large colonies will migrate to greater depths due to higher sinking velocity (or flotation velocity in spring) up to a radius of 200 μm across, but not higher.

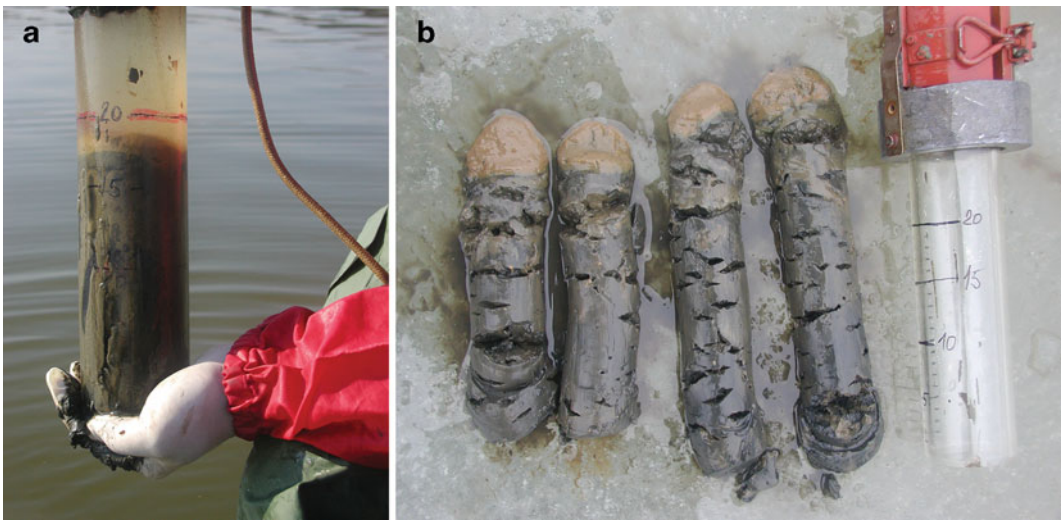

Fig. 7.6 **Sampling of sediment with *Microcystis***: (**a**) One of the sediment collection technique – sediment corer filled with sediment (**b**) Collected cores for documenting sediment structure (Photo by M. Sadílková)

Several authors have investigated changes in cell composition in late summer and early autumn. The P concentration of *M. aeruginosa* cells in late summer was found to be high (mean = 132 mmol kg^{-1} dry weight) with a cell P to lake water P concentration ratio of 10^5 (Krivtsov et al. 2005). The elemental composition remained relatively stable throughout the sampling period (July–September), with mean cell concentrations of Mg, P, S and Ca showing no significant changes. The continued high P concentration over a period of nutrient depletion in lake water is consistent with its ability of to sink to nutrient-rich lower regions of the water column. Changes in peptides related to morphological changes (termed chemotypes) were followed by Welker et al. (2007). Chemotypes that were dominant in July were no longer found after August, while two chemotypes, neither of which contained microcystins, accounted for near 80% of the colonies in November.

In addition the morphological changes of *Microcystis* population are related to events accompanying peptides (chemotypes) and molecular overturn in late summer-autumn. In the course of the season the *Microcystis* community became significantly less diverse in the planktonic, and in November two chemotypes-both of which did not contain microcystins-accounted for nearly 80% of the colonies. Some chemotypes that never accounted for high relative abundances were encountered throughout the season. In accordance with the declining % of microcystins-producing *Microcystis* colonies, the microcystins content of seston samples decreased significantly from 0.9 mg g^{-1} dry weight to levels below the detection limit Further studies are needed to establish to what extent these changes reflects seasonal changes in particular strains or shifts in the abundance of individual strains such as those assayed by Kato et al. (1991).

7.6.3 Overwintering

Microcystis overwinters as a benthic population on the lake sediment as vegetative cells rather than special akinetes (Reynolds et al. 1981; Brunberg and Bostrom 1992). Overwintering colonies can persist under various environmental conditions (Reynolds et al. 1981). No photoinhibition of photosynthesis could be observed in overwintering *Microcystis* (Rydin and Brunberg 1998). *M. aeruginosa* appeared to be more tolerant to dark anaerobic conditions than *Scenedesmus obliquus* (now *Acutodesmus obliquus*) and tolerance of these conditions may be important to the dominance of *M. aeruginosa* in eutrophic lakes (Shi et al. 2007). The benthic biomass may substantially exceed the maximum planktonic biomass (Brunberg 1995), thus indicating that *Microcystis* colonies are able to survive for long periods and accumulate at the bottom (Bostrom et al. 1989). Overwintering *Microcystis* colonies play an important role during P exchange across the sediment-water interface in eutrophic lakes because of high *Microcystis* proportion in the benthic microbial biomass (often 60–90%) (Brunberg and Blomqvist 2002). In addition a substantial part of the non-photosynthetic bacteria population (up to 40%), is associated with mucilage forming of benthic *Microcystis* colonies. Benthic *Microcystis* colonies are hot spots of enhanced bacterial activity compared to other parts of the sediment bacterial community (Bostrom et al. 1989). It is suggested that bacteria on the colony surface of *Microcystis* in sediments can be more much more important in defending the colonies than injuring them during the benthic phase of the life cycle. Bacterial production (^{3}H-thymidine incorporation) appeared to be strongly temperature-dependent, with an increasing proportion of non-growing cells in autumn and winter (Brunberg 1999).

Perhaps because of the influence of oxygen and redox conditions, marked differences in survival were found in different parts of Lake Limmeren, Sweden, by Brunberg and Blomqvist (2002). In September larger quantities of colonies (10.2×10^9 cells mL^{-1}, 1–2 m) were found in shallow bays than at deep sites (7.2×10^9 cells mL^{-1}, 6–8 m). However, in May, after the overwintering period and before the period when colonies rise into the water column, colony loss was 73% in shallow bays (2.71×10^9 cells mL^{-1}), but only 46% in deeper parts (3.32×10^9 cells mL^{-1}). Similar observations about the different ecological influences of shallow and deep sites on *Microcystis* populations have been found in Brno Reservoir, Czech Republic (Šejnohová 2008).

Analysis of the physiological and morphological conditions of benthic *M. aeruginosa* in sediments by Latour et al. (2007) involved taking colonies from the sediment surface (250 colonies mL^{-1} sediment) and also at depths of 25–35 cm (2,300 colonies mL^{-1} sediment) and 70 cm (600 colonies mL^{-1}). Environmental scanning electron microscopy (ESEM), TEM, DNA markers, cellular esterases and toxins observations showed that, as these colonies age, peripheral cells disappear, with no cells remaining in the mucilage of the deepest colonies (70 cm), an indication of the survival thresholds of the organisms. In the benthic phase, the physiological conditions (enzyme activity, cell division, and intracellular toxins) and ultrastructure (particularly the gas vesicles) of the cells surviving in the heart of the colony are comparable to those of the planktonic form, with all the potential needed for growth. Maintaining cellular integrity requires a process that can provide sufficient energy and was expressed in the reduced, but still existing, enzymatic activity that was measured, which was equivalent to a quiescent state. Overall, Wan et al. (2008) concluded that the sediment environment had negative effects on *M. aeruginosa*. The activities of catalase, glutathione peroxidase and malondialdehyde reached their highest on days 11, 6 and 6, respectively, and then dropped down markedly. The ratios of Fv/Fm and the maximal electron transfer rate declined initially, but increased again subsequently, consistent with changes in total protein. At the end of experiment, gas vacuoles were seen only seldom and the gelatinous sheath had partially disappeared. Nevertheless, upon transfer to optimal condition, the remaining population grew, though with a longer lag phase. The acute responses during the early stage of sedimentation are probably very important in aiding the long-term survival.

The occurrence of programmed cell death in colonies of *Microcystis* was indicated by a positive TUNEL reaction and by condensed regions of nucleoid DNA in Hoechst-stained material. The results suggest that senescence was a general response to adverse environmental conditions (no fungal infection), with at least 30% (minimum count) of cells in affected colonies switching to programmed cell death. The induction of cell death within individual cells occurred randomly throughout the colony (nearest-neighbour comparison to random distribution) and was unrelated to the cell cycle – since both stained and unstained cells included the full range of cell size, from dividing cells to large nondividing cells. Very few colonies of *Microcystis* were observed in the sediment-trap samples, suggesting that senescence and cell death in the planktonic population of this cyanobacterium were preceding the major phase of sedimentation (Sigee et al. 2007). After cell death, decomposition of *Microcystis* is accompanied by the release of phosphorus, during bacteria play an important role. The highest decomposition rates were recorded for the smallest size *Microcystis* fraction (<25 μm) with the addition of the sediment. The lowest decomposition rates were recorded for the smallest *Microcystis* colony fraction without the sediment, but with the addition of Gram-negative bacterial inhibitor NaN_3. The higher decomposition rates in the treatments with NaN_3 and sediment suggest that Gram-positive bacteria in the sediment are important for the decomposition process. Additionally, higher concentrations of total dissolved phosphorus (TDP) in the treatments with NaN_3 suggest that more phosphorus accumulates in the Gram-negative bacterial cells around the colony, which may be an important source of phosphorus for *Microcystis* cells. The results of this experiment suggest that both Gram-negative and Gram-positive bacteria play an important role in the decomposition of *Microcystis* cells and that the release of phosphorus for/from *Microcystis* colonies (Yuanyuan and Feizhou 2008).

Confirmation of the presence of colonies in sediments, which has usually been done using light microcopy (mostly with fluorescence), can also be done by molecular methods. These include use of primer sets for polymerase chain reaction (PCR) based on rRNA intergenic spacer analysis (Imamura et al. 2001), and DNA dependent RNA polymerase (rpoC1) and a *Microcystis* sp.-specific rpoC1 fragment (Innok et al. 2005). We expect the active development of an approach combining molecular and microscopic techniques for the detection and quantification of *Microcystis* in sediments. Standard microscopy takes considerable time and the results appear to show high variability within researchers. Molecular methods are dependent on sample preparation and the results are more difficult to interpret, but a combination can offer the reliability needed for better understanding of the benthic phase of *Microcystis*.

7.6.4 Reinvasion to the Water Column

The amount of colonies returning to the water column depends on three main factors: the total amount of colonies available, how fit these colonies are and the environmental factors influencing the sediment at the time. In a study at a

subtrophical eutrophic reservoir in China, almost 20% from the overwintering benthic stock remained in the sediment after the main period of re-invasion (Xie et al. 2003b) and such colonies could play an important role as a inoculum for subsequent recruitment, if they remain viable and are able to leave the sediment (Ihle et al. 2005). In spite of the fact that the benthic colonies are considered as a physiological rest, no morphological differences have been found between planktonic and benthic colonies (Preston et al. 1980; Trimbee and Harris 1984; Reynolds et al. 1981; Reynolds and Rogers 1976; Verspagen et al. 2004).

The onset of the *Microcystis* bloom often coincides with increasing light (Brunberg and Blomqvist 2002) and temperature (Caceres and Reynolds 1984; Walsby 1969), anoxic conditions (Trimbee and Harris 1984) low T:P ratio (Reynolds et al. 1981) and CO_2 limitation (Oliver 1994; Stahl-Delbanco et al. 2003) over lake sediments. The further potential factor regulating the recruitment of resting stages includes variations in nutrient concentrations, forms and ratios. The recruitment and growth were most pronounced at high nutrient addition (average concentrations 498 μg L^{-1} N and 134 μg L^{-1} total P) and a low N:P ratio (Ihle et al. 2005). Temperature and bioturbation have been recognized to be the most important factors for driving cyanobacteria recruitment. Other factors (such as light, nutrients, anoxia, etc.) also played a role (Tan et al. 2008). Irradiance affected the reinvasion of *Microcystis* colonies which could be established more quickly when the water is clear after the onset of stratification, allowing light to penetrate to the lake bottom (Reynolds and Bellinger 1992). *Microcystis* displays a range of variability in buoyancy in response to light which is dependent upon the previous nutrient or light history of cell (Klemer et al. 1996). The impact of these external factors is visible on internal buoyancy changes, through the amounts and densities of the gas vesicles, proteins and carbohydrates, which are designated as an active process (Brookes and Ganf 2001). In light, the photosynthetic energy is stored as carbohydrate ballast, which makes *Microcystis* colonies sink, up to the point where respiration has sufficiently reduced carbohydrate ballast to make *Microcystis* colonies buoyant again (Verspagen et al. 2004; Oliver and Ganf 2000; Vanrijn and Shilo 1985). The specific growth rate is inversely proportional to cell carbohydrate content. The growth rate is relatively high when the cell carbohydrate content is low. It can be indicated that high growth occurs when cells are buoyant, which favors blooms (Wang et al. 2010a). However, no increase in gas-vacuolation was found in colonies in light at low temperature (Thomas and Walsby 1985b; Kromkamp and Mur 1984). In the laboratory, senescent *Microcystis aeruginosa* renewed growth between 5°C and 9°C and started recruitment to the water column at 14°C, while in Lake Taihu they simultaneously grew and left the sediment surface at 9°C. However, cumulative temperatures have been recognized as the correlative factor for *Microcystis* recruitment. Because recruitment is the result of metabolic activity, we assume cumulative temperature only indirectly drove cyanobacterial recruitment, but directly promoted cyanobacterial growth to reduce ballast (Cao et al. 2008). Further laboratory investigations showed that migration of *Microcystis* from the sediments was increased by a rise in water temperature from 9°C to 15°C. The extent to which reinvasion occurred was more pronounced in the light than the dark. Other laboratory experiments have shown that *Microcystis* gets lost especially under dark conditions (Schone et al. 2010).

Because the rate of density and carbohydrate increase is not linear with time, several authors have adopted mathematical modelling to interpret changes in cyanobacterial populations (Visser et al. 1995; Thomas and Walsby 1986). In addition to active processes, passive processes resulting from resuspension by bioturbation (Wallace and Hamilton 1999, 2000; Wallace et al. 2000; Belov and Giles 1997; Howard 1997, 2001) and mixing by wind (Stahl-Delbanco and Hansson 2002) have important effects on recruitment of *Microcystis* colonies. The wind also has an important role during the short-term buoyancy by diurnal cycling of colonies with dawn/dusk migration (Verspagen et al. 2004). This is of one of the factors influencing the difference between shallow and deep sites mentioned above. Shallow areas (to 4 m) are more exposed to wind-induced mixing and light and temperature changes and therefore play an important role during the recruitment of colonies from winter to early summer (Oliver and Ganf 2000; Brookes et al. 2003). A comparison between shallow (1–2 m) and deep (6–7 m) sites showed that recruitment from the shallow bay was significantly higher over the entire season, 50% and 8%, respectively (Tsujimura et al. 2000; Verspagen et al. 2004). At a depth of 70–90 m, no seasonal variation in colony numbers was observed, although these colonies were able to grow in culture. Hence, shallow parts may be crucial by playing an important role as inoculation sites for planktonic populations (Brunberg and Blomqvist 2003) and deep areas may serve as a long-term supply, accumulating gradually each year (Brunberg and Blomqvist 2003; Verspagen et al. 2004). According to Tsujimura et al. (2000) the majority (65–85%) of the benthic population decays and the released microcystins could play a role during the reinvasion of survival colonies.

Microcystins or other peptides may also have a particular role as infochemicals during colony formation and reinvasion, though are probably important in all seasonal stages. Differences in the cellular aggregation of *M. aeruginosa* PCC 7806 and a microcystin-deficient mcyB mutant guided the discovery of a surface-exposed protein that shows increased abundance in PCC 7806 mutants deficient in microcystin production compared to the abundance of this protein in the wild type. Immunofluorescence microscopy

detected MrpC at the cell surface, suggesting an involvement of the protein in cellular interactions in strain PCC 7806. Further analyses of field samples of *Microcystis* demonstrated a strain-specific occurrence of MrpC possibly associated with distinct *Microcystis* colony types (Zilliges et al. 2008). The role of peptides as signal compounds may also be found in the shift of peptide-clones (chemotypes) in the *Microcystis* community during the course of season (Welker et al. 2007). Clones which were dominant in the plankton in July were no longer encountered after August, whereas some chemotypes that never accounted for high relative abundance were encountered throughout the season with the declining percentage of toxin-producing *Microcystis* colonies.

7.7 Conclusions

Because of its widespread occurrence globally and the frequent challenges it poses for environmental management, *Microcystis* has attracted ever-increasing attention during the past 10 years. We have attempted to summarize and interpret the ecological, taxonomic and toxicological literature. A polyphasic approach to taxonomy is now widely adopted, with an attempt to understand and distinguish different morphospecies, genotypes and chemotypes, together with their ecological consequences. The term "morphospecies" should be used for describing species within the genus. More and more studies have focused on the ecological role of extracellular and intracellular peptides and alkaloids. Although early studies of this aspect were largely to do with human toxicology, there is an increasing interest in the role of novel peptides in colony formation, population development and even signalling. All this appears to be highly relevant to understanding changes during the life cycle of *Microcystis*. Much more needs to be found about the benthic population, especially quantitative data on abundance (which should be equivalent to the plantonic population), and the ability of cells to divide and grow in the dark. The life cycle and processes associated with the various stages probably differ markedly between different lakes in temperate regions and certainly do when compared with tropical lakes, where a planktonic population may persist throughout the year.

There are many challenges for future research on *Microcystis*. For instance, there are serious gaps in understanding the relationships between morphospecies and chemotype. Can we recognize populations by morphometric parameters? Are they stable enough for robust advice about toxic or non-toxic populations? At present the results say no – only analysis of toxins or genes encoding toxins can describe the toxins produced by cyanobacterial biomass. Future research should pay much more attention to features of the whole life cycle to establish those which can help understanding of how to manage water blooms. The processes of reinvasion, colony formation and communication or signalling within populations and the whole community are some of the other promising directions for research.

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References

Ahlborn F (1895) Über die Wasserblüte *Byssus flos-aquae* und ihr Verhalten gegen Druck. Verh Naturwiss 2:25

Ahn CY, Park DK, Kim HS, Chung AS, Oh HM (2004) K: Fe ratio as an indicator of cyanobacterial bloom in a eutrophic lake. J Microbiol Biotechnol 14:290–296

Aleya L, Michard M, Khattabi H, Devaux J (2006) Coupling of the biochemical composition and calorific content of zooplankters with the *Microcystis aeruginosa* proliferation in a highly eutrophic reservoir. Environ Technol 27:1181–1190

Allen MM (1984) Cyanobacterial cell inclusions. Annu Rev Microbiol 38:1–25

Alva-Martinez AF, Sarma SSS, Nandini S (2004) Population growth of *Daphnia pulex* (Cladocera) on a mixed diet (*Microcystis aeruginosa* with *Chlorella* or *Scenedesmus*). Crustaceana 77:973–988

Anagnostidis K (1961) Untersuchungen uber die Cyanophyceen einiger Thermen in Griechenland. Institut der Systematichen Botanik und Pflanzengeographie. University of Thessaloniki, Thessaloniki, 322 pp

Anagnostidis K, Komárek J (1985) Modern approach to the classification system of cyanophytes. Introduction. Algol Stud 38(39):291–302

Babica P, Blaha L, Maršálek B (2006) Exploring the natural role of microcystins. – a review of effects on photoautotrophic organisms. J Phycol 42:1–20

Balistrieri LS, Murray JW, Paul B (1992) The cycling of iron and manganese in the water column of Lake Sammamish, Washington. Limnol Oceanogr 37:510–528

Beard SJ, Handley BA, Walsby AE (2002) Spontaneous mutations in gas vesicle genes of *Planktothrix* spp. affect gas vesicle production and critical pressure. FEMS Microbiol Lett 215:189–195

Becker S, (Matthijs HCP, van Donk E) (2010) Biotic factors in induced defence revisited: cell aggregate formation in the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806 is triggered by spent *Daphnia* medium and disrupted cells. Hydrobiologia 644:159–168. (Author citation corrected from Becker S, to Becker S, Matthijs HCP, van Donk E in Hydrobiologia erratum published in 2011)

Belikova OA (1978) Electron microscopic studies in structure of *Microcystis wesenbergii* Komárek (Cyanophyta) cells. Ukr Bot Zh 35:252–257

Belov AP, Giles JD (1997) Dynamical model of buoyant cyanobacteria. Hydrobiologia 349:87–97

Bennett EM, Carpenter SR, Caraco NF (2001) Human impact on erodable phosphorus and eutrophication: a global perspective. Bioscience 51:227–234

Bergman B, Gallon JR, Rai AN, Stal LJ (1997) N_2 fixation by non-heterocystous cyanobacteria. FEMS Microbiol Rev 19:139–185

Bickel H, Lyck S, Utkilen H (2000) Energy state and toxin content – experiments on *Microcystis aeruginosa* (Chroococcales, Cyanophyta). Phycologia 39:212–218

Biswas JK, Rana S, Bhakta JN, Jana BB (2009) Bioturbation potential of chironomid larvae for the sediment-water phosphorus exchange in simulated pond systems of varied nutrient enrichment. Ecol Eng 35:1444–1453

Boing WJ, Wagner A, Voigt H, Deppe T, Benndorf J (1998) Phytoplankton responses to grazing by *Daphnia galeata* in the biomanipulated Bautzen reservoir. Hydrobiologia 389:101–114

Bonnet MP, Poulin M (2002) Numerical modelling of the planktonic succession in a nutrient-rich reservoir: environmental and physiological factors leading to *Microcystis aeruginosa* dominance. Ecol Model 156:93–112

Bostrom B, Pettersson AK, Ahlgren I (1989) Seasonal dynamics of a cyanobacteria-dominated microbial community in surface sediments of a shallow, eutrophic lake. Aquat Sci 51:153–178

Boudreau BP, Gardiner BS, Johnson BD (2001) Rate of growth of isolated bubbles in sediments with a diagenetic source of methane. Limnol Oceanogr 46:616–622

Bowen CC, Jensen TE (1965) Blue-green algae: fine structure of the gas vacuoles. Science 147(3664):1460–1462

Bozarth CS, Schwartz AD, Shepardson JW, Colwell FS, Dreher TW (2010) Population turnover in a *Microcystis* bloom results in predominantly nontoxigenic variants late in the season. Appl Environ Microbiol 76:5207–5213

Brookes JD, Ganf GG (2001) Variations in the buoyancy response of *Microcystis aeruginosa* to nitrogen, phosphorus and light. J Plankton Res 23:1399–1411

Brookes JD, Ganf GG, Oliver RL (2000) Heterogeneity of cyanobacterial gas-vesicle volume and metabolic activity. J Plankton Res 22:1579–1589

Brookes JD, Regel RH, Ganf GG (2003) Changes in the photo-chemistry of *Microcystis aeruginosa* in response to light and mixing. New Phytol 158:151–164

Brunberg AK (1995) Microbial activity and phosphorus dynamics in eutrophic lake-sediments enriched with *Microcystis* colonies. Freshw Biol 33:541–555

Brunberg AK (1999) Contribution of bacteria in the mucilage of *Microcystis* spp. (Cyanobacteria) to benthic and pelagic bacterial production in a hypereutrophic lake. FEMS Microbiol Ecol 29:13–22

Brunberg AK, Blomqvist P (2002) Benthic overwintering of *Microcystis* colonies under different environmental conditions. J Plankton Res 24:1247–1252

Brunberg AK, Blomqvist P (2003) Recruitment of *Microcystis* (Cyanophyceae) from lake sediments: the importance of littoral inocula. J Phycol 39:58–63

Brunberg AK, Bostrom B (1992) Coupling between benthic biomass of *Microcystis* and phosphorus release from the sediments of a highly eutrophic lake. Hydrobiologia 235:375–385

Bulgakov NG, Levich AP (1999) The nitrogen: phosphorus ratio as a factor regulating phytoplankton community structure. Arch Hydrobiol 146:3–22

Caceres O, Reynolds CS (1984) Some effects of artificially enhanced anoxia on the growth of M*icrocystis aeruginosa* Kutz emend Elenkin, with special reference to the initiation of its annual growth cycle in lakes. Arch Hydrobiol 99:379–397

Canini A, Pellegrini S, Caiola MG (2003) Ultrastructural variations in *Microcystis aeruginosa* (Chroococcales, Cyanophyta) during a surface bloom induced by high incident light irradiance. Plant Biosyst 137(3):235–247

Cao HS, Kong FX, Luo LC, Shi XL, Yang Z, Zhang XF, Tao Y (2006) Effects of wind and wind-induced waves on vertical phytoplankton distribution and surface blooms of *Microcystis aeruginosa* in Lake Taihu. J Freshw Ecol 21(2):231–238

Cao HS, Tao Y, Kong FX, Yang Z (2008) Relationship between temperature and cyanobacterial recruitment from sediments in laboratory and field studies. J Freshw Ecol 23(3):405–412

Carpenter SR (2008) Phosphorus control is critical to mitigating eutrophication. Proc Natl Acad Sci USA 105(32):11039–11040

Carpenter EJ, Price CC (1976) Marine *Oscillatoria* (*Trichodesmium*) – explanation for aerobic nitrogen-fixation without heterocysts. Science 191(4233):1278–1280

Chen FZ, Xie P, Qin BQ (2007) Different competitive outcomes among four species of cladocerans under different alga combinations of colonial *Microcystis* spp. and green alga *Scenedesmus obliquus*. Hydrobiologia 581:209–215

Chen WM, Zhang QM, Dai SG (2009a) Effects of nitrate on intracellular nitrite and growth of *Microcystis aeruginosa*. J Appl Phycol 21:701–706

Chen XC, He SB, Huang YY, Kong HN, Lin Y, Li CJ, Zeng GQ (2009b) Laboratory investigation of reducing two algae from eutrophic water treated with light-shading plus aeration. Chemosphere 76:1303–1307

Chen MJ, Chen FZ, Xing P, Li HB, Wu QL (2010) Microbial eukaryotic community in response to *Microcystis* spp. bloom, as assessed by an enclosure experiment in Lake Taihu, China. FEMS Microbiol Ecol 74:19–31

Chen WM, Liu H, Zhang QM, Dai S (2011) Effect of nitrite on growth and microcystins production of *Microcystis aeruginosa* PCC 7806. J Appl Phycol 23:665–671

Chislock MF, Kaul RB, Jernigan LM, Sarnelle O, Wilson AE (2011) Abstract, aquatic sciences meeting, American Society for Limnology and Oceanography, San Juan Puerto Rico, 13–18 Feb 2011

Chu ZS, Jin XC, Iwami N, Inamori YH (2007a) The effect of temperature on growth characteristics and competitions of *Microcystis aeruginosa* and *Oscillatoria mougeotii* in a shallow, eutrophic lake simulator system. Hydrobiologia 581:217–223

Chu ZS, Jin XC, Yang B, Zeng QR (2007b) Buoyancy regulation of *Microcystis flos-aquae* during phosphorus-limited and nitrogen-limited growth. J Plankton Res 29:739–745

Coelho-Souza SA, Guimarães JRD, Mauro JBN, Miranda MR, Azevedo SMFO (2005) Mercury methylation and bacterial activity associated to tropical phytoplankton. Sci Total Environ 364:188–189

Davis TW, Berry DL, Boyer GL, Gobler CJ (2009) The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae 8(5):715–725

Deacon C, Walsby AE (1990) Gas vesicle formation in the dark, and in light of different irradiances, by the cyanobacterium *Microcystis* sp. Br Phycol J 25:133–139

Deng D, Zhang S, Li Y, Meng X, Yang W, Li Y, Li X (2010) Effects of *Microcystis aeruginosa* on population dynamics and sexual reproduction in two *Daphnia* species. J Plankton Res 32:1393–1404

Deppe T, Ockenfeld K, Meybohm A, Opitz M, Benndorf J (1999) Reduction of *Microcystis* blooms in a hypertrophic reservoir by a combined ecotechnological strategy. Hydrobiologia 409:31–38

Downing TG, Meyer C, Gehringer MM, van de Venter M (2005) Microcystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. Environ Toxicol 20:257–262

Dugdale RC, Menzel DW, Ryther JH (1961) Nitrogen fixation in the Sargasso Sea. Deep Sea Res 7:297–300

Dunton PG, Walsby AE (2005) The diameter and critical collapse pressure of gas vesicles in *Microcystis* are correlated with GvpCs of different length. FEMS Microbiol Lett 247:37–43

Ebel JP (1952) Recherches sur les polyphosphates contenus dans diverse cellules vivantes. IV. Localisation cytologique et role physiologique des polyphosphates dans la cellule vivante. Bull Soc Chim Biol Paris 34:498

Falconer IR (2005) Cyanobacterial toxins of drinking water supplies – cylindrospermopsins and microcystins. CRC Press, New York, 296 pp

Fallon RD, Brock TD (1981) Overwintering of *Microcystis* in Lake Mendota. Freshw Biol 11:217–226

Fastner J, Erhard M, von Döhren H (2001) Determination of oligopeptide diversity within a natural population of *Microcystis* spp. (Cyanobacteria) by typing single colonies by matrix-assisted laser desorption ionization -time of flight mass spectrometry. Appl Environ Microbiol 67(11):5069–5076

Fujii M, Rose AL, Omura T, Waite TD (2010) Effect of Fe(II) and Fe(III) transformation kinetics on iron acquisition by a toxic strain of *Microcystis aeruginosa*. Environ Sci Technol 44(6):1980–1986

Fujimoto N, Inamori Y, Sugiura N, Sudo R (1994) Effects of temperature-change on algal growth. Environ Technol 15(5):497–500

Fukuhara H (1987) The effect of tubificids and chironomids on particle redistribution of lake sediment. Ecol Res 2:255–264

Fulton RS, Paerl HW (1987) Toxic and inhibitory effects of the blue-green alga *Microcystis-aeruginosa* on herbivorous zooplankton. J Plankton Res 9:837–855

Furusato E, Asaeda T, Manatunge J (2004) Tolerance for prolonged darkness of three phytoplankton species, *Microcystis aeruginosa* (Cyanophyceae), *Scenedesmus quadricauda* (Chlorophyceae), and *Melosira ambigua* (Bacillariophyceae). Hydrobiologia 527:153–162

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai ZC, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320(5878):889–892

Ganf GG, Oliver RL (1982) Vertical separation of light and available nutrients as a factor causing replacement of green algae by blue-green algae in the plankton of a stratified lake. J Ecol 70:829–844

Gavel A, Maršálek B, Adámek Z (2004) Viability of *Microcystis* colonies is not damaged by silver carp (*Hypophthalmichthys molitrix*) digestion. Algol Stud 113:189–194

Ger KA, Arneson P, Goldman CR, Teh SJ (2010) Species specific differences in the ingestion of *Microcystis* cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. J Plankton Res 32:1479–1484

Ghadouani A, Pinel-Alloul B, Prepas EE (2003) Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. Freshw Biol 48:363–381

Ginn HP, Pearson LA, Neilan BA (2009) Hepatotoxin biosynthesis and regulation in cyanobacteria – the putative involvement of nitrogen and iron homeostasis mechanisms. Chiang Mai J Sci 36(2): 200–223

Gons HJ, Ebert J, Hoogveld HL, van den Hove L, Pel R, Takkenberg W, Woldringh CJ (2002) Observations on cyanobacterial population collapse in eutrophic lake water. Antonie Van Leeuwenhoek Int J Gen Mol Microbiol 81:319–326

Granéli W (1979) The influence of *Chironomus plumosus* larvae on the oxygen uptake of sediment. Arch Hydrobiol 87:385–403

Gugger M, Lyra C, Suominen I, Tsitko I, Humbert JF, Salkinoja-Salonen MS, Sivonen K (2002) Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria). Int J Syst Evol Microbiol 52:1007–1015

Gulati RD, Bronkhorst M, Van Donk E (2001) Feeding in *Daphnia galeata* on *Oscillatoria limnetica* and on detritus derived from it. J Plankton Res 23:705–718

Guseva KA (1952) Water-blooms, their causes, prognoses and eradication [In Russian]. Tr Vsesoyuz Gidrobiol Obsch 4:13–92

Gustafsson S, Hansson L-A (2004) Development of tolerance against toxic cyanobacteria in *Daphnia*. Aquat Ecol 38:37–44

Gustafsson S, Rengefors K, Hansson L-A (2005) Increased consumer fitness following transfer of toxin tolerance to offspring via maternal effects. Ecology 86:2561–2567

Hammer UT (1964) The succession of "bloom" species of blue-green algae and some causal factors. Verh Int Ver Limnol 15:829–836

Hansson LA, Gustafsson S, Rengefors K, Bomark L (2007) Cyanobacterial chemical warfare affects zooplankton community composition. Freshw Biol 52:1290–1301

Hayes PK, Walsby AE (1984) An investigation into the recycling of gas vesicle protein derived from collapsed gas vesicles. J Gen Microbiol 130:1591–1596

Holdren C, Jones W, Taggart J (2001) Chapter 7: Management techniques within the lake or reservoir. In: Holdren C, Jones W, Taggart J (eds) Managing lakes and reservoirs. North American Lake Management Society/Terrene Institute, Madison/Alexandria, pp 215–306, 400 pp

Holland DP, Walsby AE (2009) Digital recordings of gas-vesicle collapse used to measure turgor pressure and cell-water relations of cyanobacterial cells. J Microbiol Method 77:214–224

Houwink AL (1956) Flagella, gas vacuoles and cell wall structure in *Halobacterium halobium*; an electron microscopic study. J Gen Microbiol 15:146–150

Howard A (1997) Computer simulation modelling of buoyancy change in *Microcystis*. Hydrobiologia 349:111–117

Howard A (2001) Modeling movement patterns of the cyanobacterium, *Microcystis*. Ecol Appl 11:304–310

Huisman J, Hulot FD (2005) Population dynamics of harmful cyanobacteria. In: Huisman J, Matthijs HCP, Visser PM (eds) Harmful cyanobacteria, Aquatic ecology series. Springer, Dordrecht, pp 143–176, 242 pp

Huisman J, Jonker RR, Zonneveld C, Weissing FJ (1999a) Competition for light between phytoplankton species: experimental tests of mechanistic theory. Ecology 80:211–222

Huisman J, van Oostveen P, Weissing FJ (1999b) Critical depth and critical turbulence: two different mechanisms for the development of phytoplankton blooms. Limnol Oceanogr 44:1781–1787

Huisman J, van Oostveen P, Weissing FJ (1999c) Species dynamics in phytoplankton blooms: incomplete mixing and competition for light. Am Nat 154:46–68

Huisman J, Sharples J, Stroom JM, Visser PM, Kardinaal WEA, Verspagen JMH, Sommeijer B (2004) Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology 85:2960–2970

Hyenstrand P, Blomqvist P, Pettersson A (1998) Factors determining cyanobacterial success in aquatic systems – a literature review. Arch Hydrobiol Spec Issues Adv Limnol 51:41–62

Ibelings BW (1996) Changes in photosynthesis in response to combined irradiance and temperature stress in cyanobacterial surface waterblooms. J Phycol 32:549–557

Ibelings BW, Mur LR (1992) Microprofiles of photosynt hesis and oxygen concentration in *Microcystis* sp. scums. FEMS Microbiol Ecol 86:195–203

Ibelings BW, Mur LR, Walsby AE (1991) Diurnal changes in buoyancy and vertical distribution in populations of *Microcystis* in 2 shallow lakes. J Plankton Res 13:419–436

Ibelings BW, Bruning K, de Jonge J, Wolfstein K, Pires LMD, Postma J, Burger T (2005) Distribution of microcystins in a lake foodweb: no evidence for biomagnification. Microb Ecol 49:487–500

Ihle T, Jahnichen S, Benndorf J (2005) Wax and wane of *Microcystis* (Cyanophyceae) and microcystins in lake sediments: a case study in Quitzdorf Reservoir (Germany). J Phycol 41(3):479–488

Imai H, Chang KH, Kusaba M, Nakano S (2009) Temperature-dependent dominance of *Microcystis* (Cyanophyceae) species: *M. aeruginosa* and *M. wesenbergii*. J Plankton Res 31:171–178

Imamura N, Motoike I, Shimada N, Nishikori M, Morisaki H, Fukami H (2001) An efficient screening approach for anti-*Microcystis* compounds based on knowledge of aquatic microbial ecosystem. J Antibiot 54(7):582–587

Innok S, Matsumura M, Boonkerd N, Teaumroong N (2005) Detection of *Microcystis* in lake sediment using molecular genetic techniques. World J Microbiol Biotechnol 21:1559–1568

Istvanovics V, Pettersson K, Rodrigo MA, Pierson D, Padisák J (1993) *Gloeotrichia echinulata*, a colonial cyanobacterium with a unique phosphorus uptake and life strategy. J Plankton Res 15:531–552

Jacobson L, Halmann M (1982) Polyphosphate metabolism in the blue-green alga *Microcystis aeruginosa*. J Plankton Res 4:481–488

Jang M-H, Ha K, Joo GJ, Takamura N (2003) Toxin production of cyanobacteria is increased by exposure to zooplankton. Freshw Biol 48:1540–1550

Jang M-H, Jung J-M, Takamura N (2007) Changes in microcystin production in cyanobacteria exposed to zooplankton at different population densities and infochemical concentrations. Limnol Oceanogr 52(4):1454–1466

Janse I, Meima M, Kardinaal EA, Zwart G (2003) High-resolution differentiation of cyanobacteria by using rRNA-internal transcribed spacer denaturing gradient gel electrophoresis. Appl Environ Microbiol 69(11):6634–6643

Jiang HB, Qiu BS (2005) Photosynthetic adaptation of a bloom-forming cyanobacterium *Microcystis aeruginosa* (Cyanophyceae) to prolonged UV-B exposure. J Phycol 41:983–992

Jiang Y, Ji B, Wong RNS, Wong MH (2008) Statistical study on the effects of environmental factors on the growth and microcystins production of bloom-forming cyanobacterium – *Microcystis aeruginosa*. Harmful Algae 7:127–136

John KD, Huisman J, Sharples J, Sommeijer B, Visser PM, Stroom JM (2008) Summer heatwaves promote blooms of harmful cyanobacteria. Glob Change Biol 14:495–512

Jones DD, Jost M (1970) Isolation and chemical characterization of gas vacuole membranes from *Microcystis aeruginosa* Kütz. emend. Elenkin. Arch Mikrobiol 70:43–64

Jost M, Jones DD (1970) Morphological parameters and macromolecular organization of gas vacuole membranes of *Microcystis aeruginosa* Kuetz. emend. Elenkin. Can J Microbiol 16:159–164

Jost M, Zehnder A (1966) Die Gasvakuolen der Blaualge *Microcystis aeruginosa*. Schweiz Z Hydrol 28:1–3

Kaneko T, Nakajima N, Okamoto S, Suzuki I, Tanabe Y, Tamaoki M, Nakamura Y, Kasai F, Watanabe A, Kawashima K, Kishida Y, Ono A, Shimizu Y, Takahashi C, Minami C, Fujishiro T, Kohara M, Katoh M, Nakazaki N, Nakayama S, Yamada M, Tabatai S, Watanabe MM (2007) Complete genomic structure of the bloom-forming toxic cyanobacterium *Microcystis aeruginosa* NIES-843. DNA Res 14:247–256

Kato T, Watanabe MF, Watanabe M (1991) Allozyme divergence in *Microcystis* (Cyanophyceae) and its taxonomic inference. Algol Stud 64:129–140

Kilham P, Hecky RE (1988) Comparative ecology of marine and freshwater phytoplankton. Limnol Oceanogr 33:776–795

Kim SG, Joung SH, Ahn CY, Ko SR, Boo SM, Oh HM (2010) Annual variation of *Microcystis* genotypes and their potential toxicity in water and sediment from a eutrophic reservoir. FEMS Microbiol Ecol 74:93–102

Klebahn H (1895) Gasvakuolen, ein Bestandteil der Zellen der Wasserblütebildenden Phycochromaceen. Flora 80:241–282

Klebahn H (1922) Neue Untersuchengungen uber die Gasvakuolen. Jahrb Wiss Bot 61:535–589

Klemer AR, Feuillade J, Feuillade M (1982) Cyanobacterial blooms – carbon and nitrogen limitation have opposite effects on the buoyancy of *Oscillatoria*. Science 215(4540):1629–1631

Klemer AR, Cullen JJ, Mageau MT, Hanson KM, Sundell RA (1996) Cyanobacterial buoyancy regulation: the paradoxical roles of carbon. J Phycol 32:47–53

Kolkwitz R (1928) Uber Gasvakuolen bei Bakterien. Ber Dtsch Bot Ges 46:29–34

Komárek J (2010) Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept). Hydrobiologia 639:245–259

Komárek J, Anagnostidis K (1998) Cyanoprokaryota 1. Teil: Chroococcales. In: Süsswasserflora von Mitteleuropa 19/1. Fisher Verlag, Stuttgart, 548 pp

Komárek J, Komárková J (2002) Review of the European *Microcystis*-morphospecies (Cyanoprokaryotes) from nature. Czech Phycol (Fottea) 2:1–24

Komárek J, Komárková-Legnerova J, Sant'Anna CL, Azevedo MTD, Senna PAC (2002) Two common *Microcystis* species (Chroococcales, Cyanobacteria) from tropical America, including *M. panniformis* sp nov. Cryptogam Algol 23:159–177

Komárková J, Mugnai MA, Sili C, Komárek O, Turicchia S (2005) Stable morphospecies within the 16S rRNA monophyletic genus *Microcystis* (Kutzing) Kutzing. Algol Stud 117:279–295

Konopka A, Brock TD (1978) Effect of temperature on blue-green algae (cyanobacteria) in Lake Mendota. Appl Environ Microbiol 36:572–576

Konopka A, Brock TD, Walsby AE (1978) Buoyancy regulation by planktonic blue-green algae in Lake Mendota, Wisconsin. Arch Hydrobiol 83:524–537

Krivtsov V, Bellinger EG, Sigee DC (2005) Elemental composition of *Microcystis aeruginosa* under conditions of lake nutrient depletion. Aquat Ecol 39:123–134

Kromkamp J (1987) Formation and functional significance of storage products in cyanobacteria. N Z J Mar Freshw Res 21:457–465

Kromkamp J, Mur LR (1984) Buoyant density changes in the cyanobacterium *Microcystis aerugionosa* due to changes in the cellular carbohydrate content. FEMS Microbiol Lett 25:105–109

Kromkamp J, Walsby AE (1990) A computer-model of buoyancy and vertical migration in cyanobacteria. J Plankton Res 12:161–183

Kromkamp J, Konopka A, Mur LR (1988) Buoyancy regulation in light-limited continuous cultures of *Microcystis eeruginosa*. J Plankton Res 10:71–183

Kromkamp J, Vandenheuvel A, Mur LR (1989a) Phosphorus uptake and photosynthesis by phosphate-limited cultures of the cyanobacterium *Microcystis aeruginosa*. Br Phycol J 24:347–355

Kromkamp J, Vandenheuvel A, Mur LR (1989b) Formation of gas vesicles in phosphorus-limited cultures of *Microcystis aeruginosa*. J Gen Microbiol 135:1933–1939

Kurmayer R, Dittmann E, Fastner J, Chorus I (2002) Diversity of microcystin genes within a population of the toxic cyanobacterium *Microcystis* spp. in Lake Wannsee (Berlin, Germany). Microb Ecol 43:107–118

Lagauzere S, Pischedda L, Cuny P, Gilbert F, Stora G, Bonzom JM (2009) Influence of *Chironomus riparius* (Diptera, Chironomidae) and *Tubifex tubifex* (Annelida, Oligochaeta) on oxygen uptake by sediments. Consequences of uranium contamination. Environ Pollut 157:1234–1242

Lampert W (1981) Inhibitory and toxic effects of blue-green-algae on *Daphnia*. Int Rev Ges Hydrobiol 66:285–298

Lampert W (1982) Further studies on the inhibitory effect of the toxic blue-green *Microcystis aeruginosa* on the filtering rate of zooplankton. Arch Hydrobiol 95:207–220

Lampert W (1987) Laboratory studies on zooplankton-cyanobacteria interactions. N Z J Mar Freshw Res 21:483–490

Lang NJ (1968) The fine structure of the blue-green algae. Annu Rev Microbiol 22:15–46

Latour D, Salencon MJ, Reyss JL, Giraudet H (2007) Sedimentary imprint of *Microcystis aeruginosa* (Cyanobacteria) blooms in Grangent reservoir (Loire, France). J Phycol 43:417–425

Lehman EM, McDonald KE, Lehman JT (2009) Whole lake selective withdrawal experiment to control harmful cyanobacteria in an urban impoundment. Water Res 43:1187–1198

Lewandowski J, Schadach M, Hupfer M (2005) Impact of macrozoobenthos on two-dimensional small-scale heterogeneity of pore water phosphorus concentrations: in-situ study in Lake Arendsee (Germany). Hydrobiologia 549:43–55

Lewin WC, Kamjunke N, Mehner T (2003) Phosphorus uptake by *Microcystis* during passage through fish guts. Limnol Oceanogr 48(6):2392–2396

Li HL, Murphy T, Guo J, Parr T, Nalewajko C (2009) Iron-stimulated growth and microcystin production of *Microcystis novacekii* UAM 250. Limnologica 39:255–259

Lindenschmidt KE (1999) Controlling the growth of *Microcystis* using surged artificial aeration. Int Rev Hydrobiol 84:243–254

Liu Y, Tan W, Wu X, Wu Z, Yu G, Li R (2011) First report of microcystin production in *Microcystis smithii* Komárek and Anagnostidis (Cyanobacteria) from a water bloom in Eastern China. J Environ Sci 23:102–107

Lockau W, Ziegler K (2006) Cyanophycin inclusions: biosynthesis and applications. In: Rehm BHA (ed) Microbial bionanotechnology: biological self-assembly systems and biopolymer-based nanostructures. Horizon Bioscience, Norfolk, pp 79–106, 373 pp

Lukac M, Aegerter R (1993) Influence of trace metals on growth and toxin production of *Microcystis aeruginosa*. Toxicon 31:293–305

Lyra S, Suomalainen S, Gugger M, Vezie C, Sundman P, Paulin L, Sivonen K (2001) Molecular characterization of planktic cyanobacteria of *Anabaena*, *Aphanizomenon*, *Microcystis* and *Planktothrix* genera. Int J Syst Evol Microbiol 51:513–526

MacKintosh C, Beattie K, Klumpp S, Cohen C, Codd GA (1990) Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett 264:187–192

Martin-Luna B, Hernandez JA, Bes MT, Fillat MF, Peleato ML (2006a) Identification of a ferric uptake regulator from *Microcystis aeruginosa* PCC7806. FEMS Microbiol Lett 254:63–70

Martin-Luna B, Sevilla E, Hernandez JA, Bes MT, Fillat MF, Peleato ML (2006b) Fur from *Microcystis aeruginosa* binds in vitro promoter regions of the microcystin biosynthesis gene cluster. Phytochemistry 67:876–881

McCarthy MJ, James RT, Chen YW, East TL, Gardner WS (2009) Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical Lakes Okeechobee (Florida, USA) and Taihu (China). Limnology 10(3):215–227

Mercier P, Perret J (1949) Aeration of Lake Bret. Monatsbull Schweiz Ver Gas u Wasserfachm 29:25

Mermillod-Blondin F, Lemoine D, Boisson JC, Malet E, Montuelle B (2008) Relative influences of submersed macrophytes and bioturbating fauna on biogeochemical processes and microbial activities in freshwater sediments. Freshw Biol 53:1969–1982

Min X, Xu XD, Hong G, Kong RQ (2007) High variability of the gvpA-gvpC region in *Microcystis*. Prog Nat Sci 17(11):1290–1295

Mlouka A, Comte K, Castets AM, Bouchier C, Tandeau de Marsac N (2004a) The gas vesicle gene cluster from *Microcystis aeruginosa* and DNA rearrangements that lead to loss of cell buoyancy. J Bacteriol 186:2355–2365

Mlouka A, Comte K, de Marsac NT (2004b) Mobile DNA elements in the gas vesicle gene cluster of the planktonic cyanobacteria Microcystis aeruginosa. FEMS Microbiol Lett 237(1):27–34

Moisander PH, Ochiai M, Lincoff A (2009) Nutrient limitation of *Microcystis aeruginosa* in northern California Klamath River reservoirs. Harmful Algae 8:889–897

Nagai T, Imai A, Matsushige K, Fukushima T (2006) Effect of iron complexation with dissolved organic matter on the growth of cyanobacteria in a eutrophic lake. Aquat Microb Ecol 44:231–239

Nagai T, Imai A, Matsushige K, Fukushima T (2007) Growth characteristics and growth modeling of *Microcystis aeruginosa* and *Planktothrix agardhii* under iron limitation. Limnology 8:261–270

Nalewajko C, Murphy TP (2001) Effects of temperature and availability of nitrogen and phosphorus on the abundance of *Anabaena* and *Microcystis* in Lake Biwa, Japan: an experimental approach. Limnology 2:45–48

Nedzi M, Kosakowska A (2005) Influence of iron III on the growth of toxic cyanobacteria *Microcystis aeruginosa* Kutzing. In: Piecuch T (ed) VII national polish scientific conference on complex and detailed problems of environmental engineering. Wydawnictwo Uczelniane Politechn Koszalinskiej, Koszalin, pp 309–320

Nogaro G, Mermillod-Blondin F, Valett MH, Francois-Carcaillet F, Gaudet JP, Lafont M, Gibert J (2009) Ecosystem engineering at the sediment-water interface: bioturbation and consumer-substrate interaction. Oecologia 161:125–138

Oberholster PJ, Myburgh JG, Govender D, Bengis R, Botha AM (2009) Identification of toxigenic *Microcystis* strains after incidents of wild animal mortalities in the Kruger National Park, South Africa. Ecotoxicol Environ Saf 72:1177–1182

Oliver RL (1994) Floating and sinking in gas-vacuolate cyanobacteria. J Phycol 30:161–173

Oliver RL, Ganf GG (2000) Freshwater blooms. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 149–194, 669 pp

Oliver RL, Walsby AE (1984) Direct evidence for the role of light-mediated gas vesicle collapse in the buoyancy regulation of *Anabaena-Flos-Aquae* (Cyanobacteria). Limnol Oceanogr 29:879–886

Oliver RL, Thomas RH, Reynolds CS, Walsby AE (1985) The sedimentation of buoyant *Microcystis* colonies caused by precipitation with iron-containing colloid. Proc R Soc Lond B Biol 223:511–528

Onderka M (2007) Correlations between several environmental factors affecting the bloom events of cyanobacteria in Liptovska Mara reservoir (Slovakia) – a simple regression model. Ecol Model 209(2–4):412–416

Orr PT, Jones GJ (1998) Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. Limnol Oceanogr 43(7):1604–1614

Otsuka S, Suda S, Renhui L, Watanabe M, Oyaizu H, Matsumoto S, Watanabe MM (1998) 16S rDNA sequences and phylogenetic analyses of *Microcystis* strains with and without phycoerythrin. FEMS Microbiol Lett 164:119–124

Otsuka S, Suda S, Li RH, Matsumoto S, Watanabe MM (2000) Morphological variability of colonies of *Microcystis* morphospecies in culture. J Gen Appl Microbiol 46:39–50

Otsuka S, Suda S, Shibata S, Oyaizu H, Matsumoto S, Watanabe MM (2001) A proposal for the unification of five species of the cyanobacterial genus *Microcystis* Kutzing ex Lemmermann 1907 under the rules of the bacteriological code. Int J Syst Evol Microbiol 51:873–879

Ou MM, Wang Y, Zhou BX, Cai WM (2006) Effects of iron and phosphorus on *Microcystis* physiological reactions. Biomed Environ Sci 19:399–404

Paerl HW (1983) Partitioning of CO_2 fixation in the colonial cyanobacterium *Microcystis aeruginosa*: mechanism promoting formation of surface scums. Appl Environ Microbiol 46:252–259

Paerl HW, Bland PT, Bowles ND, Haibach ME (1985) Adaptation to high-intensity low-wavelength light among surface blooms of the cyanobacterium *Microcystis aeruginosa*. Appl Environ Microbiol 49:1046–1052

Parra OO, Ugarte E, Mora S, Libreman M, Aron A, Balabanoff L (1980) Remarks on a bloom of *Microcystis aeruginosa* Kutzing. Nova Hedwig 33:971–990

Paterson MJ, Findlay DL, Salki AG, Hendzel LL, Hesslein RH (2002) The effects of *Daphnia* on nutrient stoichiometry and filamentous cyanobacteria: a mesocosm experiment in a eutrophic lake. Freshw Biol 47:1217–1233

Preston T, Stewart WDP, Reynolds CS (1980) Bloom-forming cyanobacterium *Microcystis-aeruginosa* overwinters on sediment surface. Nature 288(5789):365–367

Rabouille S, Salencon MJ, Thebault JM (2005) Functional analysis of *Microcystis* vertical migration: a dynamic model as a prospecting tool I – processes analysis. Ecol Model 188:386–403

Redfield A (1958) The biological control of chemical factors in the environment. Am Sci 46:205–221

Reynolds CS (1999) Non-determinism to probability, or N: P in the community ecology of phytoplankton. Arch Hydrobiol 146:23–35

Reynolds CS (2006) Ecology of phytoplankton. Cambridge University Press, Cambridge, 507 pp

Reynolds CS, Bellinger EG (1992) Patterns of abundance and dominance of the phytoplankton of Rostherne Mere, England – evidence from an 18-year data set. Aquat Sci 54:10–36

Reynolds CS, Rogers DA (1976) Seasonal variations in the vertical distribution and buoyancy of *Microcystis aeruginosa* Kütz. emend. Elenkin in Rostherne Mere, England. Hydrobiologia 48:17–23

Reynolds CS, Walsby AE (1975) Water-blooms. Biol Rev 50:437–481

Reynolds CS, Jaworski GHM, Cmiech HA, Leedale GF (1981) On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kütz emend Elenkin. Proc R Soc Lond B Biol 293(1068):419–477

Rhee GY (1980) Continuous culture in phytoplankton ecology. Adv Aquat Microbiol 2:151–303

Robarts RD, Zohary T (1987) Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming Cyanobacteria. N Z J Mar Freshw Res 21:391–399

Rohrlack T, Christoffersen O, Kaebernick M, Neilan BA (2004) Cyanobacterial protease inhibitor microviridin J causes a lethal molting disruption in *Daphania*. Appl Environ Microbiol 70:5047–5050

Romo S, Soria J, Fernandez F, Ouahid Y, Baron-Sola A (2012) Water residence time and the dynamics of toxic cyanobacteria. Freshw Biol. doi:10.1111/j.1365-2427.2012.02734.x

Rueter JG, Petersen RR (1987) Micronutrient effects on cyanobacterial growth and physiology. N Z J Mar Freshw Res 21:435–445

Rydin E, Brunberg AK (1998) Seasonal dynamics of phosphorus in Lake Erken surface sediment. Arch Hydrobiol Spec Issues Adv Limnol 51:157–167

Sabour B, Loudiki M, Vasconcelos V (2009) Growth responses of *Microcystis ichthyoblabe* Kutzing and *Anabaena aphanizomenoides* Forti (Cyanobacteria) under different nitrogen and phosphorus conditions. Chem Ecol 25:337–344

Sanchis D, Padilla C, Del Campo FF, Quesada A, Sanz-Alferez S (2005) Phylogenetic and morphological analyses of *Microcystis* strains (Cyanophyta/Cyanobacteria) from a Spanish water reservoir. Nova Hedwig 81:431–448

Sarnelle O, Wilson AE (2005) Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. Limnol Oceanogr 50:1565–1570

Sarnelle O, Gustafsson S, Hansson L-A (2010) Effects of cyanobacteria on fitness components of the herbivore *Daphnia*. J Plankton Res 32:471–477

Schatz D, Keren Y, Vardi A, Sukenik A, Carmeli S, Borner T, Dittmann E, Kaplan A (2007) Towards clarification of the biological role of microcystins, a family of cyanobacterial toxins. Environ Microbiol 9(4):965–970

Schindler DW (1977) Evolution of phosphorus limitation in lakes. Science 165:260–262

Schone K, Jahnichen S, Ihle T, Ludwig F, Benndorf J (2010) Arriving in better shape: benthic *Microcystis* as inoculum for pelagic growth. Harmful Algae 9:494–503

Sedmak B, Elersek T (2005) Microcystins induce morphological and physiological changes in selected representative phytoplanktons. Microb Ecol 50:298–305

Šejnohová L (2008) *Microcystis*. New findings in peptide production, autecology and taxonomy by cyanobacterium *Microcystis*. Ph.D. thesis, Institute of Botany, Czech Academy of Sciences, Brno, 178 pp, ISBN 978-80-86188-27-0. www.sejnohova.cz/soubory/sejnohova_PhD_tisk080709.pdf

Šejnohová L, Harustiakova D, Welker M, Maršálek B (2011) Morphology and oligopeptides composition by the genus *Microcystis* – is there any possible relationship? (unpublished data)

Semyalo R, Rohrlack T, Larsson P (2009) Growth and survival responses of a tropical *Daphnia* (*Daphnia lumholtzi*) to cell-bound microcystins. J Plankton Res 31:827–835

Shao JH, Wu XQ, Li RH (2009) Physiological responses of *Microcystis aeruginosa* PCC7806 to nonanoic acid stress. Environ Toxicol 24:610–617

Shapiro J (1990) Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH. Verh Int Ver Limnol 24:38–54

Shen H, Song LR (2007) Comparative studies on physiological responses to phosphorus in two phenotypes of bloom-forming *Microcystis*. Hydrobiologia 592:475–486

Shi XL, Yang LY, Niu XJ, Xiao L, Kong ZM, Qin BQ, Ga G (2003) Intracellular phosphorus metabolism of *Microcystis aeruginosa* under various redox potential in darkness. Microbiol Res 158:345–352

Shi XL, Kong FX, Yu Y, Yang Z (2007) Survival of *Microcystis aeruginosa* and *Scenedesmus obliquus* under dark anaerobic conditions. Mar Freshw Res 58:634–639

Shi LM, Cai YF, Wang XY, Li PF, Yu Y, Kong FX (2010) Community structure of bacteria associated with *Microcystis* colonies from cyanobacterial blooms. J Freshw Ecol 25:193–203

Sigee DC, Selwyn A, Gallois P, Dean AP (2007) Patterns of cell death in freshwater colonial cyanobacteria during the late summer bloom. Phycologia 46:284–292

Sirenko A (1972) Physiological fundamentals of reproduction of the blue-green algae in reservoirs [in Russian]. Naukova Dumka, Kiev, p 403

Sleytr UB, Messner P, Pum D, Sara M (1988) Crystalline bacterial cell surface layers. Springer, Berlin, 193 pp

Šmajs D, Šmarda J (1999) New findings of S-layers among cyanobacteria. Algol Stud 94:317–332

Šmajs D, Šmarda J, Krzyzanek V (1999) A comparative study of fine structure of cyanobacterial gas vesicles. Algol Stud 94:305–316

Šmarda J (1991) S-layer of chroococcal cell walls. Algol Stud 64:41–51

Šmarda J, Šmajs D (1996) Morphological types of gas vesicles. Algol Stud 83:485–499

Smith VH (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green-algae in lake phytoplankton. Science 221(4611): 669–671

Smith RV, Peat A (1967) Comparative structure of the gas vacuoles of blue-green algae. Arch Mikrobiol 57:111–122

Sommaruga R, Chen Y, Liu Z (2009) Multiple strategies of bloom-forming *Microcystis* to minimize damage by solar ultraviolet radiation in surface waters. Microb Ecol 57:667–674

Sommer U (1985) Comparison between steady-state and non-steady state competition – experiments with natural phytoplankton. Limnol Oceanogr 30(2):335–346

Stahl-Delbanco A, Hansson LA (2002) Effects of bioturbation on recruitment of algal cells from the "seed bank" of lake sediments. Limnol Oceanogr 47(6):1836–1843

Stahl-Delbanco A, Hansson LA, Gyllstrom M (2003) Recruitment of resting stages may induce blooms of *Microcystis* at low N:P ratios. J Plankton Res 25(9):1099–1106

Stoeckenius W, Kunau WH (1968) Further characterization of particulate fractions from lysed cell envelopes of *Halobacterium halobium* and isolation of gas vacule membranes. J Cell Biol 38:336–357

Stoeckenius W, Rowen R (1967) A morphological study of *Halobacterium halobium* and its lysis in media of low salt concentration. J Cell Biol 34:355–395

Strayer RF, Tiedje JM (1978) In situ methane production in a small, hypereutrophic, hard-water lake: loss of methane from sediments by vertical diffusion and ebullition. Limnol Oceanogr 23(6):1201–1206

Stumm W, Morgan JJ (1996) Aquatic chemistry, chemical equilibria and rates in natural waters, 3rd edn. Wiley, New York, 1022 pp

Svensson JM, Enrich-Prast A, Leonardson L (2001) Nitrification and denitrification in a eutrophic lake sediment bioturbated by oligochaetes. Aquat Microb Ecol 23(2):177–186

Takeya K, Kuwata A, Yoshida M, Miyazaki T (2004) Effect of dilution rate on competitive interactions between the cyanobacterium *Microcystis novacekii* and the green alga *Scenedesmus quadricauda* in mixed chemostat cultures. J Plankton Res 26:29–35

Tan X, Kong FX, Cao HS, Yu Y, Zhang M (2008) Recruitment of bloom-forming cyanobacteria and its driving factors. Afr J Biotechnol 7(25):4726–4731

Tan X, Kong F, Zeng Q, Cao H, Qian S, Zhang M (2009) Seasonal variation of *Microcystis* in Lake Taihu and its relationships with environmental factors. J Environ Sci 21:892–899

Tandeau de Marsac N, Houmard J (1993) Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol Rev 104:119–190

Thomas RH, Walsby AE (1985a) Regulation of buoyancy by the cyanobacterium *Microcystis*. Br Phycol J 20:191–197

Thomas RH, Walsby AE (1985b) Buoyancy regulation in a strain of *Microcystis*. J Gen Microbiol 131:799–809

Thomas RH, Walsby AE (1986) The effect of temperature on recovery of buoyancy by *Microcystis*. J Gen Microbiol 132: 1665–1672

Toetz DW (1981) Effects of whole lake mixing on water quality and phytoplankton. Water Res 15(10):1205–1210

Trimbee AM, Harris GP (1984) Phytoplankton population dynamics of a small reservoir – use of sedimentation traps to quantify the loss of diatoms and recruitment of summer bloom-forming blue-green-algae. J Plankton Res 6:897–918

Trimbee AM, Prepas EE (1988) The effect of oxygen depletion on the timing and magnitude of blue-green algal blooms. Verh Int Ver Limnol 23:220–223

Tsujimura S, Tsukada H, Nakahara H, Nakajima T, Nishino M (2000) Seasonal variations of *Microcystis* populations in sediments of Lake Biwa, Japan. Hydrobiologia 434:183–192

Utkilen H, Gjolme N (1995) Iron-stimulated toxin production in *Microcystis aeruginosa*. Appl Environ Microbiol 61:797–800

Van Donk E (2006) Food-web interactions in lakes – what is the impact of chemical information conveyance? In: Dicke M, Takken W (eds) Chemical ecology: from gene to ecosystem, vol 16, Wageningen Ur Frontis series. Springer, Dordrecht, pp 145–160

Van Donk E (2007) Chemical information transfer in freshwater plankton. Ecol Inform 2:112–120

Van Donk E, Cerbin S, Wilken S, Helmsing NR, Ptacnik R, Verschoor AM (2009) The effect of a mixotrophic chrysophyte on toxic and colony-forming cyanobacteria. Freshw Biol 54:1843–1855

Van Gremberghe I, Vanormelingen P, Van der Gucht K, Souffreau C, Vyverman W, De Meester L (2009a) Priority effects in experimental populations of the cyanobacterium *Microcystis*. Environ Microbiol 11:2564–2573

Van Gremberghe I, Vanormelingen P, Vanelslander B, Van der Gucht K, D'Hondt S, De Meester L, Vyverman W (2009b) Genotype-dependent interactions among sympatric *Microcystis* strains mediated by *Daphnia* grazing. Oikos 118:1647–1658

Van Wichelen J, Van Gremberghe I, Vanormelingen P, Debeer AE, Leporcq B, Menzel D, Codd GA (2010) Strong effects of amoebae grazing on the biomass and genetic structure of a *Microcystis* bloom (Cyanobacteria). Environ Microbiol 12:2797–2813

Vanrijn J, Shilo M (1985) Carbohydrate fluctuations, gas vacuolation, and vertical migration of scum-forming cyanobacteria in fishponds. Limnol Oceanogr 30:1219–1228

Varis O (1993) Cyanobacteria dynamics in a restored Finnish lake – a long-term simulation study. Hydrobiologia 268:129–145

Verspagen JMH, Snelder E, Visser PM, Huisman J, Mur LR, Ibelings BW (2004) Recruitment of benthic *Microcystis* (Cyanophyceae) to the water column: internal buoyancy changes or resuspension? J Phycol 40:260–270

Via-Ordorika L, Fastner J, Kurmayer R, Hisbergues M, Dittmann E, Komárek J, Erhard M, Chorus I (2004) Distribution of microcystin-producing and non-microcystin-producing *Microcystis* sp. in European freshwater bodies: detection of microcystins and microcystin genes in individual colonies. Syst Appl Microbiol 27:592–602

Visser PM, Ibelings BW, Mur LR (1995) Autumnal sedimentation of *Microcystis* spp. as result of an increase in carbohydrate ballast at reduced temperature. J Plankton Res 17:919–933

Visser PM, Ibelings BW, van der Veer B, Koedood J, Mur LR (1996a) Artificial mixing prevents nuisance blooms of the cyanobacterium *Microcystis* in Lake Nieuwe Meer, the Netherlands. Freshw Biol 36:435–450

Visser PM, Ketelaars HAM, van Breemen L, Mur LR (1996b) Diurnal buoyancy changes of *Microcystis* in an artificially mixed storage reservoir. Hydrobiologia 331:131–141

Visser PM, Passarge J, Mur LR (1997) Modelling vertical migration of the cyanobacterium *Microcystis*. Hydrobiologia 349:99–109

Visser PM, Ibelings BW, Mur LR, Walsby AE (2005) The ecophysiology of the harmful cyanobacterium *Microcystis*. In: Huisman J, Matthijs HCP, Visser PM (eds) Harmful cyanobacteria, Aquatic ecology series. Springer, Dordrecht, pp 109–142, 242 pp

Waaland JR, Branton D (1969) Gas vacuole development in a blue-green alga. Science 163(3873):1339–1341

Wallace BB, Hamilton DP (1999) The effect of variations in irradiance on buoyancy regulation in *Microcystis aeruginosa*. Limnol Oceanogr 44:273–281

Wallace BB, Hamilton DP (2000) Simulation of water-bloom formation in the cyanobacterium *Microcystis aeruginosa*. J Plankton Res 22:1127–1138

Wallace BB, Bailey MC, Hamilton DP (2000) Simulation of vertical position of buoyancy regulating *Microcystis aeruginosa* in a shallow eutrophic lake. Aquat Sci 62:320–333

Walsby AE (1969) The permeability of blue-green algal gas-vacuole membranes to gas. Proc R Soc Lond B Biol 173:235–255

Walsby AE (1971) The pressure relationships of gas vacuoles. Proc R Soc Lond B Biol 178:301–326

Walsby AE (1972) Structure and function of gas vacuoles. Bacteriol Rev 36(1):1–32

Walsby AE (1980) The water relations of gas-vacuolate prokaryotes. Proc R Soc Lond B Biol 208:78–102

Walsby AE (1984) Lower limit of the gas permeability coefficient of gas vesicles. Proc R Soc Lond B Biol 223:177–196

Walsby AE (1994) Gas vesicles. Microbiol Rev 58:94–144

Walsby AE, Bleything A (1988) The dimensions of cyanobacterial gas vesicles in relation to their efficiency in providing buoyancy and withstanding pressure. J Gen Microbiol 134:2635–2645

Walsby AE, Hayes PK (1988) The minor cyanobacterial gas vesicle protein, Gvpc, is attached to the outer surface of the gas vesicle. J Gen Microbiol 134:2647–2657

Walsh K, Jones GJ, Dunstan RH (1997) Effect of irradiance on fatty acid, carotenoid, total protein composition and growth of *Microcystis aeruginosa*. Phytochemistry 44:817–824

Walsh K, Jones GJ, Dunstan RH (1998) Effect of high irradiance and iron on volatile odour compounds in the cyanobacterium *Microcystis aeruginosa*. Phytochemistry 49:1227–1239

Wan N, Tang J, Li QM, Song LR (2008) The response of *Microcystis* to sediment environments and the assessment for its overwintering – a simulation study in a novel device. Fresenius Environ Bull 17:2146–2151

Wang YY, Chen FZ (2008) Decomposition and phosphorus release from four different size fraction of *Microcystis* spp. taken from Lake Taihu, China. J Environ Sci 20:891–896

Wang H, Gruden CL, Bridgeman TB, Chaffin JD (2009) Detection and quantification of *Microcystis* spp. and microcystin-LR in Western Lake Erie during the summer of 2007. Water Sci Technol 60:1837–1846

Wang C, Kong HN, He SB, Zheng XY, Li CJ (2010a) The inverse correlation between growth rate and cell carbohydrate content of *Microcystis aeruginosa*. J Appl Phycol 22:105–107

Wang C, Kong HN, Wang XZ, Wu HD, Lin Y, He SB (2010b) Effects of iron on growth and intracellular chemical contents of *Microcystis aeruginosa*. Biomed Environ Sci 23:48–52

Wang W, Liu Y, Yang Z (2010c) Combined effects of nitrogen content in media and *Ochromonas* sp grazing on colony formation of cultured *Microcystis aeruginosa*. J Limnol 69:193–198

Wang XD, Qin BQ, Gao G, Paerl HW (2010d) Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. J Plankton Res 32:457–470

Webster IT (1990) Effect of wind on the distribution of phytoplankton cells in lakes. Limnol Oceanogr 35:989–1001

Webster IT, Hutchinson PA (1994) Effect of wind on the distribution of phytoplankton cells in lakes revisited. Limnol Oceanogr 39:365–373

Welker M, von Dohren H (2006) Cyanobacterial peptides – nature's own combinatorial biosynthesis. FEMS Microbiol Rev 30:530–563

Welker M, Maršálek B, Šejnohová L, von Dohren H (2006) Detection and identification of oligopeptides in *Microcystis* (cyanobacteria) colonies: toward an understanding of metabolic diversity. Peptides 27(9):2090–2103

Welker M, Šejnohová L, Nemethova D, Maršálek B (2007) Seasonal shifts in chemotype composition of *Microcystis* sp. communities in the pelagial and the sediment of a shallow reservoir. Limnol Oceanogr 52:609–619

White SH, Duivenvoorden LJ, Fabbro LD (2005) Impacts of a toxic *Microcystis* bloom on the macroinvertebrate fauna of Lake Elphinstone, Central Queensland, Australia. Hydrobiologia 548:117–126

Wiegand C, Pflugmacher S (2005) Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. Toxicol Appl Pharmacol 203:201–218

Wood S, Dietrich DR, Cary CS, Hamilton DP (2012) Increasing Microcystis cell density enhanced microcystin synthesis: a mesocosm study. Inland Waters 2:17–22

Worm J, Sondergaard M (1998) Dynamics of heterotrophic bacteria attached to *Microcystis* spp. (Cyanobacteria). Aquat Microb Ecol 14(1):19–28

Wu XD, Kong FX (2009) Effects of light and wind speed on the vertical distribution of *Microcystis aeruginosa* colonies of different sizes during a summer bloom. Int Rev Hydrobiol 94:258–266

Wu WJ, Li GB, Li DH, Liu YD (2010) Temperature may be the dominating factor on the alternant succession of *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* in Dianchi lake. Fresenius Environ Bull 19:846–853

Wyatt JT, Silvey JKG (1969) Nitrogen fixation by *Gloeocapsa*. Science 165(3896):908–909

Xie LQ, Xie P, Li SX, Tang HJ, Liu H (2003a) The low TN:TP ratio, a cause or a result of *Microcystis* blooms? Water Res 37(9):2073–2080

Xie LQ, Xie P, Tang HJ (2003b) Enhancement of dissolved phosphorus release from sediment to lake water by *Microcystis* blooms – an enclosure experiment in a hyper-eutrophic, subtropical Chinese lake. Environ Pollut 122:391–399

Xing W, Huang WM, Liu GH, Liu YD (2008) Effects of iron on physiological and biochemical characteristics of *Microcystis wesenbergii* (Kom.) Kom. (Cyanobacterium). Fresenius Environ Bull 17(12):2034–2042

Xu H, Paerl HW, Qin BQ, Zhu GW, Gao G (2010) Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnol Oceanogr 55(1):420–432

Yagi O, Ohkubo N, Tomioka N, Okada M (1994) Effect of Irradiance and temperature on photosynthetic activity of the cyanobacterium *Microcystis* spp. Environ Technol 15(4):389–394

Yamamoto Y (2010) Contribution of bioturbation by the red swamp crayfish *Procambarus clarkii* to the recruitment of bloom-forming cyanobacteria from sediment. J Limnol 69(1):102–111

Yamamoto Y, Nakahara H (2005) Competitive dominance of the cyanobacterium *Microcystis aeruginosa* in nutrient-rich culture conditions with special reference to dissolved inorganic carbon uptake. Phycol Res 53:201–208

Yamamoto Y, Shiah F-K (2010) Variation in the growth of *Microcystis aeruginosa* depending on colony size and position in colonies. Ann Limnol Int J Limnol 46:47–52

Yamamoto Y, Tsukada H (2009) Measurement of in situ specific growth rates of M*icrocystis* (cyanobacteria) from the frequency of dividing cells. J Phycol 45:1003–1009

Yamamoto Y, Shiah F-K, Chen Y-L (2011) Importance of large colony formation in bloom-forming cyanobacteria in eutrophic ponds. Ann Limnol Int J Limnol 47:167–174

Yan H, Pan G, Zou H, Song LR, Zhang MM (2004) Effects of nitrogen forms on the production of cyanobacterial toxin microcystin-LR by an isolated *Microcystis aeruginosa*. J Environ Sci Heal A 39(11–12): 2993–3003

Yang Z, Kong F, Shi XL, Cao H (2006) Morphological response of *Microcystis aeruginosa* to grazing by different sorts of zooplankton. Hydrobiologia 563:225–230

Yang Z, Kong FX, Shi XL, Zhang M, Xing P, Cao HS (2008) Changes in the morphology and polysaccharide content of *Microcystis aeruginosa* (Cyanobacteria) during flagellate grazing. J Phycol 44: 716–720

Ye LL, Wu XD, Tan XA, Shi XL, Li DM, Yu Y, Zhang M, Kong FX (2010) Cell lysis of cyanobacteria and its implications for nutrient dynamics. Int Rev Hydrobiol 95:235–245

Yuan L, Zhu W, Xiao L, Yang LY (2009) Phosphorus cycling between the colonial cyanobacterium *Microcystis aeruginosa* and attached bacteria, *Pseudomonas*. Aquat Ecol 43(4):859–866

Yuanyuan W, Feizhou C (2008) Decomposition and phosphorus release from four different size fractions of *Microcystis* spp. taken from Lake Taihu, China. J Environ Sci 20(7):891–896

Zeng SJ, Yuan XZ, Shi XS, Qiu YL (2010) Effect of inoculum/substrate ratio on methane yield and orthophosphate release from anaerobic digestion of *Microcystis* spp. J Hazard Mater 178:89–93

Zevenboom W, Mur LR (1984) Growth and photosynthethic response of the cyanobacterium *Microcystis aeruginosa* in relation to photoperiodicity and irradiance. Arch Microbiol 129:61–66

Zhang M, Kong FX, Wu XD, Xing P (2008a) Different photochemical responses of phytoplankters from the large shallow Taihu Lake of subtropical China in relation to light and mixing. Hydrobiologia 603:267–278

Zhang X, Xie P, Huang X (2008b) A review of nontraditional biomanipulation. Sci World J 8:1184–1196

Zhu GW, Qin BQ, Gao G (2005) Direct evidence of phosphorus outbreak release from sediment to overlying water in a large shallow lake caused by strong wind wave disturbance. Chin Sci Bull 50(6):577–582

Zilliges Y, Kehr J-C, Mikkat S, Bouchier C, Tandeau de Marsac N, Börner T, Dittmann E (2008) An extracellular glycoprotein is implicated in cell-cell contacts in the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806. J Bacteriol 190(8):2871–2879

Freshwater Picocyanobacteria: Single Cells, Microcolonies and Colonial Forms

8

Cristiana Callieri, Gertrud Cronberg
and John G. Stockner

Contents

Abbreviations

Pcy	picocyanobacteria
CPcy	colonial picocyanobacteria
PE-rich	phycoerythrin containing Pcy
PC-rich	phycocyanin containing Pcy
Chl	chlorophyll *a*
HNF	heterotrophic nanoflagellates
ITS-1	internal transcribed spacer region between the 16S rRNA and 23S rRNA genes
T-RFLP	terminal restriction fragment length polymorphism
DGGE	denaturating gradient gel electrophoresis
ARISA	automated ribosomal intergenic spacer analyses
DCM	deep chlorophyll maximum
OTU	operational taxonomic unit
RT-qPCR	real-time quantitative polymerase chain reaction
FDC	frequency of dividing cells
RUBISCO	ribulose-1,5-bisphosphate carboxylase oxygenase
ELF	enzyme labelled fluorescence
APA	extracellular phosphatase activity
DOP	dissolved organic phosphorus
CPD	cyclobutane pyrimidine dimer
BWF	biological weighting functions
MAAs	mycosporine-like amino acid compounds
K_d	extinction coefficient of photosynthetically active radiation

Summary

This chapter deals with some taxonomic and ecological aspects of picocyanobacteria (Pcy) single-cells, microcolonies and other colonial (CPcy), that are common in lakes throughout the world, and abundant across a wide spectrum of trophic conditions. We discuss phenotypic diversity of Pcy in conjunction with a genotypic approach in order

C. Callieri (✉)
National Research Council, Institute of Ecosystem Study,
Largo Tonolli 50, 28922 Verbania Pallanza, Italy
e-mail: c.callieri@ise.cnr.it

G. Cronberg
Aquatic Ecology, Ecology Building, University of Lund,
BOX 65, S-22100 Lund, Sweden
e-mail: gertrud.cronberg@biol.lu.se

J.G. Stockner
Eco-Logic Ltd., 2624 Mathers Avenue, West Vancouver,
BC, Canada V7V 2J4
e-mail: jestock@shaw.ca

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to resolve whether a similar morphology also reflects a phylogenetic relationship. Microcolonies of different size (from 5 to 50 cells) constitute a gradient without a net separation from single-celled types and should be considered Pcy, as transition forms from single-cell to colonial morphotypes. The single-celled Pcy populations tend to be predominant in large, deep oligo-mesotrophic lakes, while the CPcy find optimal conditions in warmer, shallower and more nutrient rich lakes. The knowledge of Pcy diversity in pelagic and littoral zone habitats is a key to understand the dominance of certain genotypes in the water column and of their ubiquity. An anlysis is included of the factors (biotic and abiotic) influencing the dynamics of the different Pcy forms.

8.1 Introduction

A phylogenomic study of the evolution of cyanobacterial traits shows that the earliest lineages were probably unicellular cells in terrestrial and/or freshwater environments (Sánchez-Baracaldo et al. 2005; Blank and Sánchez-Baracaldo 2010) rather than in the marine habitat as suggested by Honda et al. (1999). This discovery opens new prospects for the study of freshwater Pcy and provides an impetus for phylogenetic and ecological investigations to clarify the many uncertainties in the literature. One of the most striking differences between freshwater and marine Pcy lies in the extraordinary richness of morphotypes and the unresolved phylogeny of Pcy in lakes. However, despite marked phylogenetic differences, Pcy have a similar pattern in their absolute and relative importance in freshwater and marine systems along the trophic gradient (Bell and Kalff 2001).

This chapter discusses freshwater Pcy single-cells together with microcolonies and other colonial forms, that are common in lakes throughout the world (Hawley and Whitton 1991a) and abundant across a wide spectrum of trophic conditions. Though their abundance in meso-eutrophic lakes is often high enough to reduce transparency and cause water discolouration, they seldom create the blooms associated with larger colonial cyanobacteria. Although studies of the ecology of microcolonies and colonial forms are few, there have been sufficient studies within the past 25 year of Pcy in lakes and their role in food webs to warrant synthesis and further review (Stockner et al. 2000; Callieri 2008).

The picocyanobacteria exhibit two common morphologies: single cells (coccoid, rods) and colonies with diverse colonial morphology. We propose here to consider microcolonies as transitional forms from single cells to colonial morphotypes (Fig. 8.1). Under favourable conditions some Pcy can develop mucilage or a sheath and remain near to the mother cell forming a clump. Here we designate as picocyanobacteria (Pcy) the single cells (0.2–2.0 μm) which are the major component of the picophytoplankton community, and as colonial picocyanobacteria (CPcy) all species whose predominant morph is colonial and have single cells ranging from 0.5 to 3.0 μm. Microcolonies of different size (from 5 to 50 cells) constitute a gradient without a clear separation from the single-celled type and should be considered Pcy.

Until the past few decades most research on Pcy focused on the CPcy, because their colonies were easily seen by conventional microscopy, and their ubiquity in meso-eutrophic lakes of Northern Europe attracted the attention of early descriptive taxonomists (Lemmermann 1904; Naumann 1924; Skuja 1948). CPcy also seem to have received more thorough systematic descriptions, along with some comment on habitat preference and distribution (Komárek 1958, 1996; Cronberg 1991; Komárková-Legnerová and Cronberg 1994).

The emergence of Pcy as an important research topic for limnologists and oceanographers provides an opportunity to discuss to what extent this large and diverse group share a common ecology. The current challenge is to understand better the relationship between the diversity and ecology of Pcy, microcolonies and CPcy and their interaction with the environmental factors that allow the proliferation of the most competitive genotypes. The study of genome divergence, lateral gene transfer and genomic islands will provide new opportunities for a better understanding of niche adaptation (Dufresne et al. 2008; Scanlan et al. 2009 and Chap. 20). We conclude this review with a plea for limnologists to pay more attention to these organisms. Their extent and abundance in lakes may provide an important message about the changes likely to occur in pelagic community structure in the warmer world expected in the near future (Mann 1993; Stockner 1998).

8.2 Taxonomy and Phylogenetic Diversity

8.2.1 Single Cells and Microcolonies

Even more than for most prokaryotes the morphological features of Pcy are insufficiently distinct to provide a reliable basis for discriminating taxa (Staley 1997; Komárek et al. 2004). The criteria for the definition of genera of single-celled Pcy such as *Cyanobium*, *Synechococcus* and *Cyanothece dianae/cedrorum*-type (Komárek 1996) have been supplanted by molecular methods which focus on clade divergence in the phylogenetic tree rather than on morphological differences. The clade containing Pcy (*Synechococcus/Prochlorococcus/Cyanobium* sensu Sánchez-Baracaldo et al. 2005) is formed by coccoid and rod-shaped cells with a diameter <3 μm. Analysis of 16S ribosomal DNA (rDNA) of freshwater *Synechococcus* shows it is a polyphyletic genus and cannot be considered a natural taxon (Urbach et al. 1998; Robertson et al. 2001). In the phylogenetic tree the Antarctic strains represent a unique and highly adapted clade related only peripherally to *Synechococcus* sp. (Cluster 5.2, Marine Cluster B) (Vincent et al. 2000; Powell et al. 2005).

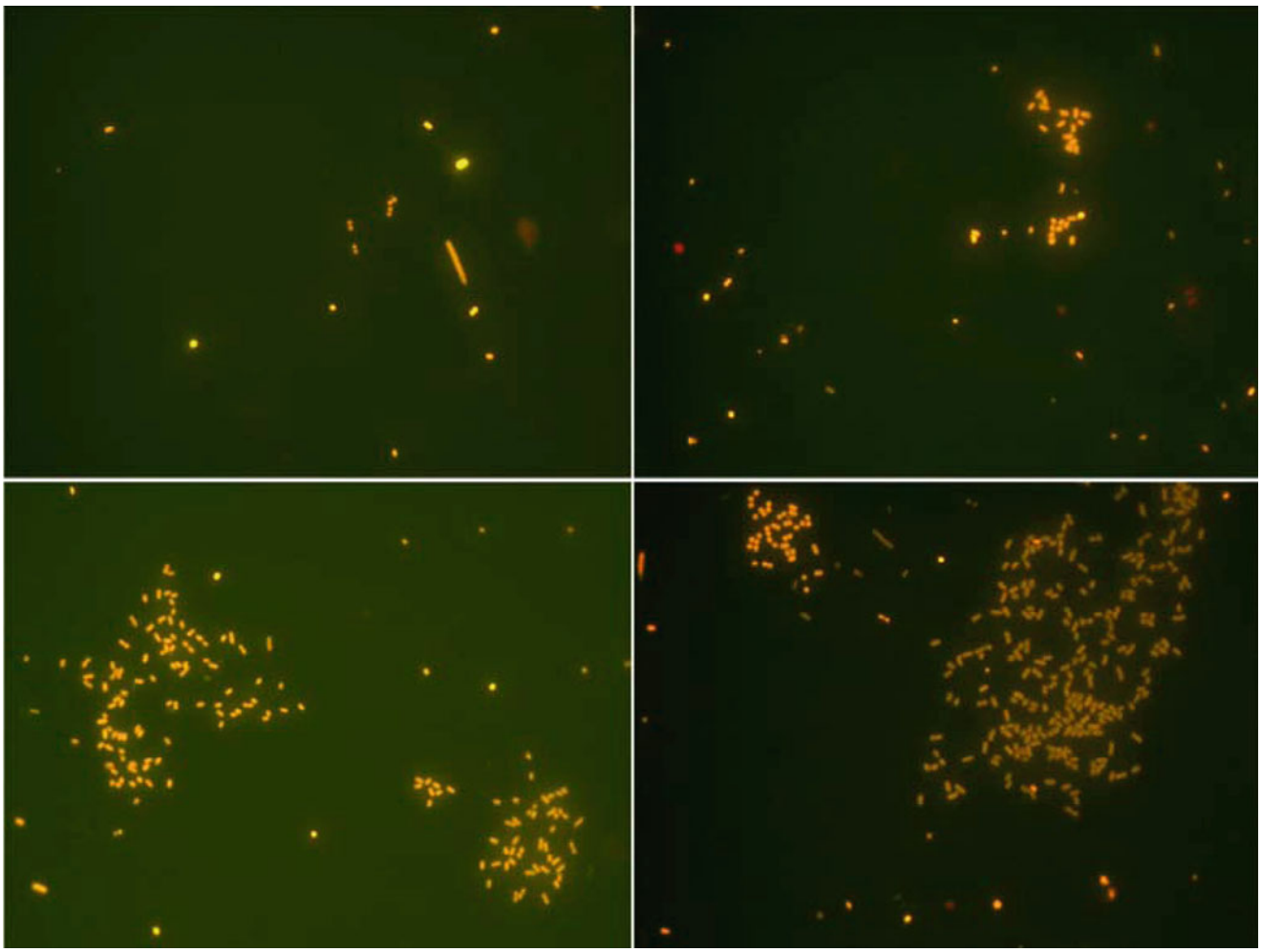

Fig. 8.1 Single cells, microcolonies and larger colonies in Lake Maggiore, a large deep oligo-mesotrophic subalpine lake in northern Italy. PE-rich Pcy under epifluorescence microscopy (1250x) (blue-excitation)

It has become increasingly clear that phenotypic diversity should be evaluated in conjunction with genotypic analysis in order to resolve whether a similar morphology also reflects a phylogenetic relationship (Wilmotte and Golubić 1991). Even though many genetically distinct *Synechococcus* strains have been found (Robertson et al. 2001), it is still helpful to classify Pcy into two cell types: the first with yellow autofluorescing phycoerythrin (PE-rich cells), and the second with red autofluorescing phycocyanin (PC-rich cells) as the major light-harvesting pigment (Fig. 8.2) (Wood et al. 1985; Ernst 1991). Phycoerythrin-rich strains have an absorption peak at ~560 nm, and hence absorb green light effectively. Phycocyanin-rich strains have an absorption peak at ~625 nm, and absorb orange-red light effectively (Callieri et al. 1996a; Haverkamp et al. 2008).

Phylogenetic studies have mostly been performed using sequence data derived from 16S rDNA which is a conserved gene, but shows high pair-wise similarity in freshwater Pcy (Fig. 8.3, Crosbie et al. 2003a) and cannot resolve the actual genetic variation that accompanies their physiological diversity (Urbach et al. 1998). Less conserved genetic markers can offer a more detailed definition of the diversity of Pcy (Haverkamp et al. 2009). In particular the spacer between the 16S and 23S rDNA (ITS-1) exhibits a great deal of length and sequence variation and can be used to differentiate marine and freshwater Pcy ecotypes using fingerprinting techniques (T-RFLP, DGGE or ARISA) (Rocap et al. 2002; Becker et al. 2002; Ernst et al. 2003). In a glacial Andean lake system (Argentina) the ARISA showed habitat specificity of some Pcy OTU, emphasizing the microdiversity due to geographical barriers (Caravati et al. 2010). Similarly, the study of functional genes as, for example, those encoding for phycocyanin and phycoerythrin (*cpc*BA and *cpe*BA) can offer another perspective on the evolution of Pcy, grouping the strains on the basis of pigment composition (Haverkamp et al. 2008; Jasser et al. 2011). Indeed, it has been found that phylogenies based on phycobiliprotein rod gene components are not congruent with the 16S rRNA phylogeny whilst those based on the allophycocyanin core are congruent (Six et al. 2007; Haverkamp et al. 2009). This is explained

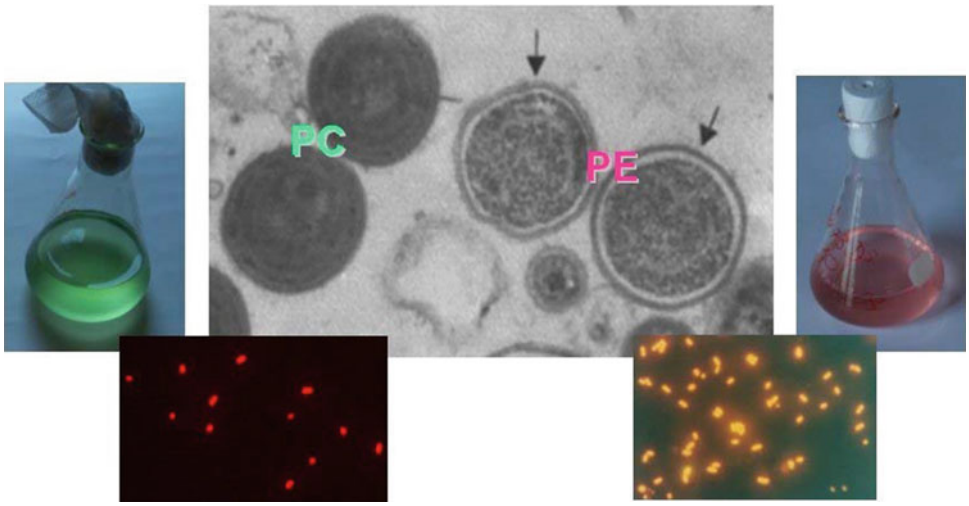

Fig. 8.2 Three different views of Pcy: liquid cultures (*green*: PC-cells, *pink*: PE-cells); epifluorescence under blue excitation with PC cells (showing in *red*) and PE cells (showing in *yellow*); transmission electron microscope photo (showing the different internal structure of PC- and PE- cells)

by the presence of phycobilisome rod components within genomic islands that potentially allow their transfer between *Synechococcus* lineages (Six et al. 2007). A comparison of marine *Synechococcus* showed that their adaptation to different ecological niches can be related to the variable number of horizontally acquired genes located in highly variable genomic islands (Dufresne et al. 2008). Similar to what happens in *Prochlorococcus* (Coleman et al. 2006); it is likely that phages carrying host genes can mediate lateral gene transfer. This discovery opens new perspectives to the understanding of the local adaptation and the definition of species within the *Synechococcus* group.

The phylogenetic approach combined with RT-qPCR has been used to assess Pcy in both marine (Ahlgren et al. 2006) and freshwater community structure (Becker et al. 2000, 2007). Using small subunit (ssu) rDNA sequences from novel culture isolates together with environmental samples from the Baltic Sea and seven freshwater lakes, Sánchez-Baracaldo et al. (2008) showed that freshwater Pcy communities encompass much greater diversity than is found in marine systems. They hypothesised a more rapid speciation in lakes allowed by geographical barriers and noticed that most of the sequences from the Baltic Sea were related closely to freshwater lineages.

To provide a more realistic phylogenetic tree of cyanobacteria Sánchez-Baracaldo et al. (2005) used a combination of different molecular sequence data instead of individual genes. Flanking a selection of morphological traits into the backbone cyanobacterial tree they showed that the ancestral cyanobacterium were a single cell and that filamentous/colonial forms appeared later in time. The presence of a well-defined sheath, associated with the colonial lifestyle, is a trait which has been lost and attained several times during evolution. In Arctic lakes the Pcy strains isolated appear to be closely related to *Microcystis elabens* (Vincent et al. 2000) now reclassified as a species of *Aphanothece* (Komárek and Anagnostidis 1999). Thus, it is tempting to suggest that microcolonies, which are frequently found in freshwater, may be considered transition forms from single cells to true colonial. In this sense the investigations done by Crosbie et al. (2003a) confirm the existence of single cell/single colony strains, with different degrees of aggregation, possibly belonging to the group H and group B subalpine cluster I.

8.2.2 Colonies

Most of the small pico-cell-sized CPcy belong to the chroococcal cyanobacteria. The cell size ranges between 0.5 and 3 μm in diameter and the cell form is generally spherical, ovoid or rodlike. The cells occur in colonies of different morphology and these colonial morphologies are often species specific, and can be used in discriminating species. The cells inside the CPcy colony can be loosely or densely packed, or can form pseudofilaments or other net-like structures. In some species the cells are attached to mucilaginous stalks, which are centred in the middle of the colony (e.g. *Cyanonephron*, *Snowella*). In lakes where CPcy are common, there is usually a mixture of species each with distinctive colony structure, which are quite readily identifiable. CPcy are found throughout the entire spectrum of lake trophic conditions; however most tend to occur in more productive meso-eutrophic lakes. Some of the most common CPcy in fresh waters are species belonging to the genera *Aphanocapsa, Aphanothece, Chroococcus, Coelosphaerium, Cyanobion, Cyanodictium, Merismopedia, Romeria, Snowella* and *Tetracercus* (Table 8.1).

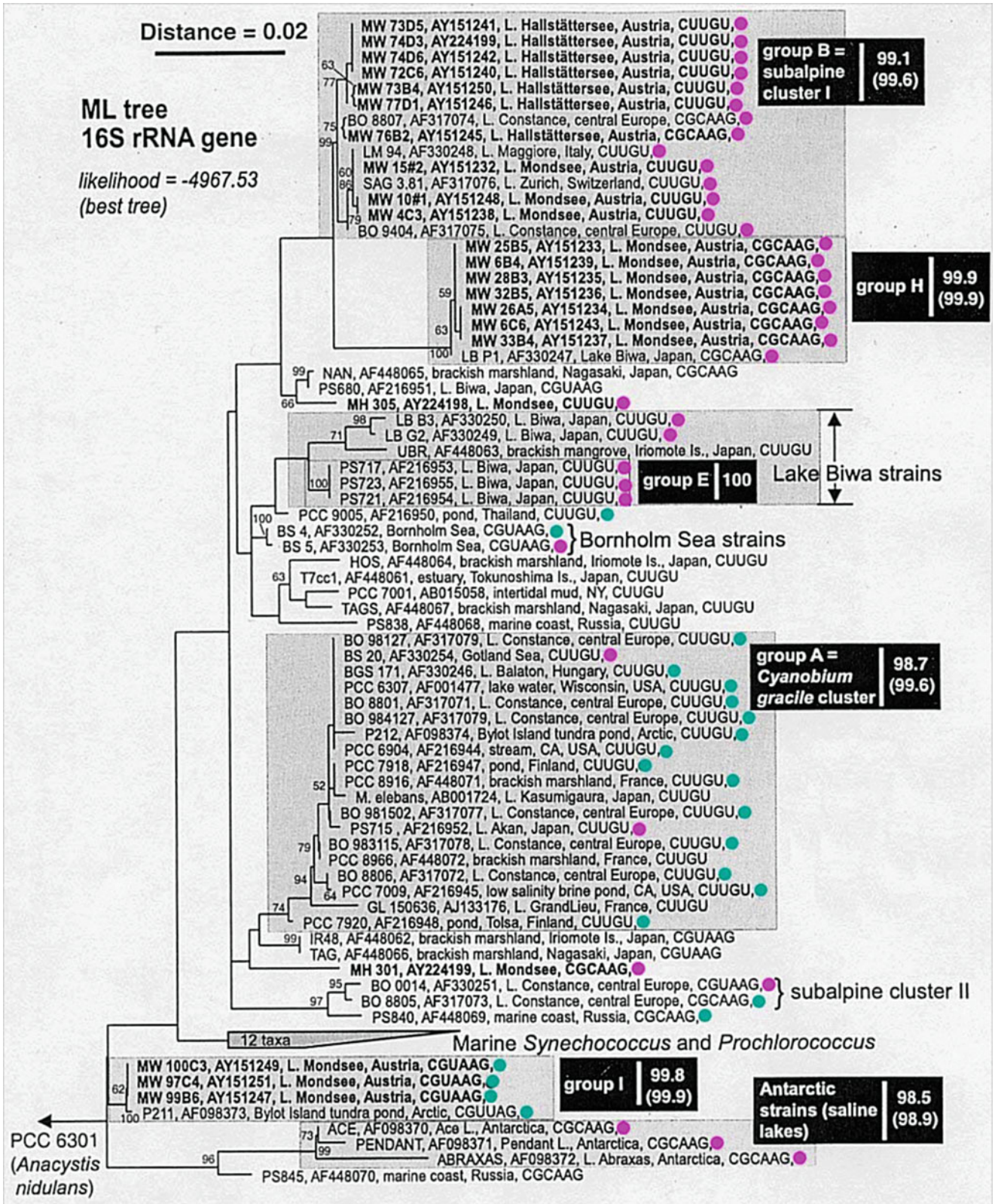

Fig. 8.3 Maximum-likelihood phylogenetic tree of SSU rDNA sequences from unicellular picocyanobacteria, (green circle: PC-rich, pink circle: PE-rich) (For details see Crosbie et al. 2003a, reproduced with permission)

Table 8.1 Examples of well described planktonic picocyanobacteria

Species	Colony structure	Single cell shape	Cell dimensions, μm	Ecological niche	Country	References
Aphanocapsa conferta (W.& G.S. West) Komárková-Legnerová & Cronberg	Colonies planktonic, spherical or irregular, up to 80 μm diam. ± densely packed cells, mucilage delicate, colourless, indistinct at margin	Spherical	1.5–2(2.4)	Eutrophic lakes, temperate zone (tropical questioned)	Temperate zone	Komárková-Legnerová and Cronberg (1994)
Aphanocapsa delicatissima W. & G. S. West	Spherical to irregular colonies in diffuse mucilage, <50 μm. Cells evenly spread in colony	Spherical	0.5–1	Probably cosmopolitan, in eutrophic lakes	Temperate zone, England, Norway, Sweden, North America	Komárková-Legnerová and Cronberg (1994)
A. elegans (Lemmermann) Joosten	Colonies planktonic, spherical, ellipsoid with evenly and densely distributed cells. Bright blue-green cells without gas vesicles	Spherical or oval	(1.7)–2–3	Eutrophic lakes and ponds	Temperate zone, The Netherlands	Joosten (2006)
A. incerta (Lemmermann) Cronberg & Komárek	Colonies spherical to irregular sometimes flattened. Cells densely, irregularly packed in the colony	Spherical	0.5–2 (2.7)	Eutrophic lakes and ponds	Cosmopolitan	Cronberg and Komárek (1994)
A. holsatica (Lemmermann) Cronberg & Komárek	Colonies irregularly shaped, clathrate with cells ± densely aggregated.	Spherical	About 1	Eutrophic lakes and ponds	Cosmopolitan, very common in Denmark, Sweden, Finland, Germany	Cronberg and Komárek (1994)
A. elachista W. & G. S. West	Colonies spherical to oval, few-celled. Cells solitary or in pairs, sparsely positioned	Spherical	1.5–1.8 (2)	Eutrophic waters	Tropical distribution, in temperate zone during summer	West and West (1894)
A. marina Hansgirg 1890	Colonies small, formless, gelatinous greyish-blue, sometimes united into a dark blue-green mass	Spherical	0.4–0.5	Marine and brackish, in pools with salty water, on wet rocks	Atlantic coasts of Europe, Mediterranean Sea	Komárek and Anagnostidis (1998)
A. nubilum Komárek & Kling	Colonies irregular with cells ± densely packed	Spherical	1.2–1.5	Mesotrophic lakes	Africa, cosmopolitan	Komárek and Kling (1991)
A. planctonica (G. M. Smith) Komárek & Anagnostidis	Colonies irregular with cells sparsely distributed	Spherical	2–3	Oligo-to eutrophic lakes	N. America, Europe, temperate zone	Komárek and Kling (1991)
Aphanothece bachmannii Komárková-Legnerová & Cronberg	Colonies usually flat, clathrate with elongate cells in ± parallel row	Elongate, oval to cylindrical	0.5–1 × 0.8–2	Freshwater and brackish water, meso- to eutrophic lakes and ponds	Temperate zones, Denmark, Finland, Sweden, Baltic Sea, N. Germany,	Komárková-Legnerová and Cronberg (1994)
A. clathrata W. & G. S. West	Colonies irregular, large, flat, clathrate with cells ± evenly distributed	Rod-like, straight or slightly curved	0.5–0.7–1 × 2.5–3.5–4	Freshwater and brackish water, oligo-eutrophic lakes and ponds	Cosmopolitan, common in Finland, Sweden, Germany, Baltic Sea	Komárková-Legnerová and Cronberg (1994)

A. parallelliformis Cronberg	Colonies microscopic, planktonic, elongated or irregular, 10–75 × 8–20 µm, with cells in rows, which are more or less packed parallel in colourless mucilage	Cells are rodshaped to cylindrical with cut ends, pale blue-green without gas vesicles	1.5–3.2 × 0.9–1.4	Mesotrophic	Baltic Sea, brackish water	Cronberg (2003)
A. pseudoglebulenta Joosten	Colonies planktonic, 50–100 µm, composed of closely packed ellipsoid or spherical subcolonies with loosely distributed cells	Spherical or subspherical to rodlike cells with rounded ends, without gas vesicles	1–1.6–(2.4) × 1–1.2	Fishpond	Sassenhein N, near Haren, Gronigen, Netherlands	Joosten (2006)
A. smithii Komárková-Legnerová & Cronberg	Colonies spherical to oval, sometimes elongated, of varying size. Cells densely packed.	Short cylindrical to oval	1–1.2–1.5 × 1.8–2.2–3.5	Freshwater, oligo- to eutrophic lakes and ponds	Canada, Finland, Sweden, N. Germany	Komárková-Legnerová and Cronberg (1994)
Chroococcus microscopicus Komárková-Legnerová & Cronberg	Colonies cloud-like with cells regularly arranged in groups of 4–6 cells, surrounded by sheath	Spherical or hemispherical after division	0.7–1	Meso- to eutrophic lakes	Known only from Sweden, but probably wider distribution	Komárková-Legnerová and Cronberg (1994)
C. aphanocapsoides Skuja	Colonies spherical, oval or irregular, size up to 100 µm diam. with cells gathered in groups of 2–8 cells	Spherical to hemispherical after division, with individual sheath	1.8–2	Oligo- to eutrophic freshwater lakes	Known only from Sweden, but probably wider distribution	Skuja (1964)
C. minimus (Keissler) Lemmermann	Colonies with 2–8 cells. Cells regularly arranged in groups within colourless mucilage	Spherical to hemispherical after division, ± individual sheath	1.7–3	Oligo- to mesotrophic lakes	North temperate zone	Cronberg and Komárek (1994)
Coelosphaerium minutissimum Lemmermann	Colonies planktonic, spherical to oval, 20–30 (170) µm diam. with cells just beneath the colony surface	Spherical	0.8–1.2	Oligo- slightly eutrophic waters, also brackish waters	Northern Europe, including Baltic Sea	Komárek and Komárková-Legnerová (1992)
C. subarcticum Komárek & Komárková-Legnerová	Colonies spherical or oval, sometimes as two hemi-spherical colonies. Cells regularly arranged in ± one layer near colony surface	Spherical	1.2–1.6	In oligo- to eutrophic waters	Temperate zone	Komárek and Komárková-Legnerová (1992)
Cyanocatena imperfecta (Cronberg & Weibull) Joosten	Net-like colonies of small, loose pseudofilaments	Spherical, just before division slightly elongate, precipitate ferric rings	0.4–0.8.–1	Planktonic in eutrophic lakes	Germany, Greece, Sweden, Canada, E. African lakes, Laos	Joosten (2006)
Cyanocatena planctonica Hindák	Colonies irregular, spherical, oval with hyaline mucilage, <20 µm in diam.	Cells cylindrical to oval with ring-like precipitates on the surface	0.5–1.2 × 1–2.5	Planktonic eutrophic ponds and lakes	Austria, Czech Republic, Germany Greece, Slovakia	Hindák (1975)

(continued)

Table 8.1 (continued)

Species	Colony structure	Single cell shape	Cell dimensions, µm	Ecological niche	Country	References
Cyanodictyon balticum Cronberg	Colonies microscopic, spherical or irregular spherical with several subcolonies, up to 175 µm diam, mucilage hyaline	Rodshaped to oval, pale blue-green in colour. The cells are arranged in rows in a superficial layer of the colonies	1.7–2.7 × 1	Planktonic in mesotrophic to eutrophic ponds or lakes, also in the Baltic Sea	North temperate zone	Cronberg (2003)
Cyanodictyon filiforme Komárková-Legnerová & Cronberg	Filaments unbranched sometimes forming loose net-like bundles	Rod-like cells in short filaments	0.2–0.5 × 1.5–2.5	Freshwater, planktonic, meso- and eutrophic lakes	Sweden, Canada	Komárková-Legnerová and Cronberg (1994)
C. planctonicum Meyer	Colonies 3-dimensional and irregular elongated net-like, <150 µm	Oval to almost rod-like	0.8–1 × 1.5	Freshwater, planktonic in eutrophic lakes	Denmark, Sweden, N. Germany	Meyer (1994)
C. reticulataum (Lemmermann) Geitler	Colonies initially formed by rows of single cells, older ones net-like, 3-dimensional	Spherical	1–1.5	Freshwater, meso-eutrophic lakes	Austria, Denmark, Germany, Greece, Russia, Canada	Komárková-Legnerová and Cronberg (1994)
C. tubiforme Cronberg	Colonies with uniserial to multiserial rows of cells, sometimes clathrate, <400 µm diam.	Spherical, hemispherical after division, or widely oval to rod-shaped	1.9–2.2 × 2.2–3.8	Freshwater, planktonic in shallow eutrophic lakes	Sweden, the Baltic states, N. Germany	Cronberg (1988)
Cyanogranis ferruginea (Wawrik) Hindák	Colonies with 3–50–(100) cells, <12 µm in diam.	Spherical to oval with black ferric precipitate	0.4–1 × 0.6–1.5	Planktonic in freshwaters, fish ponds, slightly alkaline	Austria, Czech Republic, Germany Greece, Slovakia	Hindák (1982)
Cyanonephron styloides Hickel	Spherical to ellipsoid colonies with cells sitting on stalks	Cells elongate, kidney-shaped	0.8–1.2 × 2.3–4.5	Freshwater, brackish water, meso- to hypertrophic lakes	Finland, Sweden, N. Germany, Baltic Sea	Hickel (1985)
Lemmermanniella parva Hindák	Spherical colonies with cells beneath the surface, 55–120 (180) µm in diam.	Cells short cylindrical or oval	0.8–1 × 1–1.5 (1.8)	Freshwater, brackish water, meso-eutrophic	Slovakia, Baltic Sea	Hindák (1985)
L. pallida (Lemmermann) Geitler	Spherical colonies, with cells beneath the surface, <85 (138) in diam.	Rod-like, cylindrical	0.5–1.6 × (0.7) 1.1–3.7 (4.3)	Freshwater, brackish water, planktonic meso- to eutrophic lakes	Sweden, Denmark, N. Germany, Finland, Russia, the Baltic Sea	Komárková and Cronberg (1985)
Merismopedia warmingiana Lagerheim	Colonies regular, flat. Cells grouped in quadrates, slightly irregular, 4–16 (64) cells in colony	Spherical to slightly elongate before division	0.5–1 (1.2)	Eutrophic, polluted waters also in brackish and saline ponds, sometimes in masses	Cosmopolitan, Europe	Lagerheim (1883)
M. tenuissima Lemmermann	Colonies flat, ± rectangular. Cells grouped in quadrates, regular, 16–100 cells per colony	Spherical or oval, after division hemispherical	0.4–1.6–(2–2.5?)	Eutrophic, stagnant freshwaters, fish ponds, in brackish waters	Cosmopolitan, common in Europe	Komárková-Legnerová and Cronberg (1994)
Pannus punctiferus (Komárek & Komárková-Legnerová) Joosten	Colonies spherical to subspherical, up to 80 µm diam.; cells just beneath colony surface	Spherical	1–1	Tropical or warmer regions, mesotrophic lakes	Canada, Africa	Joosten (2006)

Pannus spumosus Hickel	Colonies ± spherical, clathrate. Cells densely arranged with individual fine sheath	Spherical	1–1.5	From brackish stagnant waters	N. Germany, Sweden, Baltic Sea	Hickel (1991)
Romeria caruaru Komárek et al.	Cells in short irregular trichomes, 1–4 cells in the rows	Cells short cylindrical with rounded ends without gas vesicles	1.1–2.8 × 0.7–1.0	Eutrophic dams	Caruaru Dam, Brazil in toxic cyanobacterial blooms	Komárek et al. (2001)
Romeria elegans (Wołoszynska) Koczwara	Trichomes 20–45 µm long, easily fragmented into single cells, without mucilage	Cylindrical, slightly bent cells with rounded ends	1–2 × 3.5–7	Common in eutrophic waters, also in brackish water	Temperate zone, also in the Baltic Sea	Geitler (1932)
R. leopoliense (Raciborski) Koczwara	Trichomes with 4–6 cells, twisted in half circles, no mucilage sheath	Elongated cells, slightly bent with cut ends	0.8–1.2 × 3–5	Common in eutrophic waters	Temperate zone	Geitler (1932)
Snowella atomus Komárek & Hindák	Colonies planktonic, ± spherical, up to 25 µm diam. Cells on outer end of mucilaginous stalks joined at centre of colony	Spherical, after division hemispherical	0.6–1.4	Mesotrophic lakes	Central Europe	Komárek and Komárková-Legnerová (1992)
Synechococcus bacillaris Butcher	Cells solitary or forming short chains	Spherical, oval to cylindrical	(1.5)–1.7–4.5	Plankton in reservoirs, seawater	England	Butcher (1952)
S. gaarderi Ålvik	Cells solitary or in pairs	Spherical to ellipsoid	1.2–1.5 × 1.5–2.8	Plankton in brackish and marine waters close to the coast	Norway	Ålvik (1934)
S. elongatus Nägeli	Cells solitary or in clusters, sometimes making small chains	cells oval to cylindrical, straight or slightly curved	1–2–3 × 2–9	Mostly subaerophytic	Temperate zone	Komárek (1976)
Synechococcus nidulans (Pringsheim) Komárek	Cells solitary, sometimes mass-devlopment	Rod-like without mucilage	0.4–1.3–2.2 × 1.5–8.5	Freshwater, pools and small ponds, eutrophic	Temperate zone	Bourrelly (1985)
S. plancticus Drews et al.	Cells solitary	Oval to rod-like	0.9–1.1 × 1.5–3	Plankton , freshwater, wastewater ponds	Czech Republic. Germany	Drews et al. (1961)
Synechococcus sp. sensu Waterbury et al.	Cells solitary	Spherical, oval to rod-like, red-coloured	0.8–1.7 × 1.8–2.2	Plankton, marine	Pacific, Northern Gulf Stream, Sargasso Sea, Arabian Gulf	Waterbury et al. (1986)
Tetrarcus ilsteri Skuja	Colonies with 2–4 cells in ring-like groups dispersed in colourless mucilage, <150 µm diam.	Cells half-moon formed with yellow-green to blue-violet colour	1–1.5 × 2.5–4	Humic, oligotrophic lakes and ponds	Lithuania, Finland, Sweden, Switzerland	Skuja (1932)

There are few records of the development of CPcy in nature or in culture and accordingly the available literature is sparse. The CPcy are only a small component in water blooms and are thus often overlooked and usually neglected. Due to their minute cell size, unfiltered samples must be taken from lakes, as the CPcy will otherwise pass through the meshes of normal plankton nets (mesh width 10–45 μm) and are lost. The colonies of CPcy are normally counted in sedimentation chambers under 400–1,000× magnification. The settling time of CPcy must be long; for example in a 5 cm long chamber the settling time should be about 4 days and nights and the sample must be well preserved in Lugol's solution.

Bláha and Marsálek (1999) isolated seven strains of Pcy from water blooms in the Czech Republic and tested their ability to produce toxins. They found that two strains produced the hepatotoxin microcystin. As most all Pcy can readily pass through the filters in drinking water treatment plants, they cannot be neglected and as possible sources of toxins should be carefully monitored.

Several studies of cyanobacteria within Brazil's drinking water reservoirs have been made and provide an overview of their current status (Domingos et al. 1999; Komárek et al. 2001; Sant'Anna et al. 2004; Furtado et al. 2009). As most of the reservoirs were dominated by cyanobacteria, it was important to assess for cyanobacterial toxicity. Cyanobacteria, including CPcy and Pcy were isolated and tested for algal toxins with HPLC and/or ELISA methods. Several cyanobacteria including CPcy were found to be toxic; for example *Aphanocapsa cumulus* from Caruaru Reservoirs, where several persons have died after having dialysis with drinking water from these reservoirs (Domingos et al. 1999). In Tabocas reservoir CPcy were also very frequent and strains were isolated, e.g. *Aphanocapsa cumulus, Aphanothece stratus*, and a new species *Romeria carauru* was recorded (Komárek et al. 2001). No toxin tests were made on these species, thus it was not confirmed whether these species were responsible for the Carauro (Komárek et al. 2001), but they likely were. The morphology of strains of *A. stratus* and *Cyanobium*-like Pcy were studied with TEM, and their morphologies were identical and they evidently belonged to the same species. During the life-cycle *A. stratus* lived in colonies in the benthos, but as single cells or microcolonies in the plankton (Komárek et al. 2001). Pairwise similarities 16S rDNA sequence of *A. stratus, R. carauru* and other related cyanobacterial strains were carried out, but further studies are needed to evaluate genotype resemblance.

Furtado et al. (2009) studied cyanobacteria in waste stabilization pond systems in Brazil and recorded several CPcy. Morphological identification and phylogenetic analysis based on 16S rDNA sequence were made on the isolated strains and a phylogenetic tree with related species was obtained. They noted that the strains *Synchococcus* CENA 108 and *Merismopedia* CENA 206 were both capable of producing microcystins at large population densities ($>1 \times 10^6$ cells mL^{-1}, Furtado et al. 2009).

8.3 A Common Ecology?

8.3.1 Single Cell and Microcolony Dynamics

Sufficient information is now available to define the different patterns of Pcy, single-cells and microcolonies, in lakes of different size, thermal regime and trophic state (Callieri 2008, 2010). In lakes of temperate regions maxima generally conform to a typical bimodal pattern, with a spring or early summer peak and a second peak during summer-autumn (Stockner et al. 2000). This is the case of most of the subalpine large lakes without ice-cover e.g. Lake Maggiore, Lake Constance, but also for Lake Stechlin, a deep oligo-mesotrophic lake in the Baltic Lake District where ice-cover occurs (Padisák et al. 2003). However looking at the long-term series of Pcy abundance in Lake Maggiore (Fig. 8.4) (Callieri and Piscia 2002), Lake Constance (Gaedke and Weisse 1998), and Lake Stechlin (Padisák et al. 2003) not all the years are clearly bimodal. The great interannual variability of Pcy dynamics is mainly related to differences in weather conditions which cause different spring mixing regimes and timing of water column stabilization (Weisse 1993). Studies in British Columbia's temperate oligotrophic lakes have shown a clear trend in both magnitude and timing of Pcy seasonal maxima related to levels and duration of seasonal nutrient supplementation (Stockner and Shortreed 1988, 1994).

Large spring peaks are also common in eutrophic, hypereutrophic and dystrophic shallow lakes (Sime-Ngando 1995; Jasser 1997; Mózes et al. 2006). The seasonal patterns found in Danish lakes (Søndergaard 1991), Canadian lakes (Pick and Agbeti 1991), Lake Biwa, Japan (Maeda et al. 1992), English lakes (Hawley and Whitton 1991b; Sánchez-Baracaldo et al. 2008), Lake Mondsee, Austria (Crosbie et al. 2003c), Lakes Bourget and Geneva, France (Personnic et al. 2009a) all lack the spring peak, there being only a summer or autumn maximum. The lack of Pcy spring peak in these was probably due to weak stratification in March-April and hence relatively deep vertical mixing. This interpretation is strengthened by studies on Lake Baikal, where owing to winter ice cover and extended spring isothermal conditions, Pcy reach high abundance only in summer months and lack a spring peak (Belykh et al. 2006).

In Arctic and Antarctic lakes Pcy are widely distributed, despite the fact they are generally present in low abundance in the marine polar environment (Vincent 2000; Chap. 13). In continental Antarctica in meromictic saline Ace Lake Pcy reached concentrations one order of magnitude higher

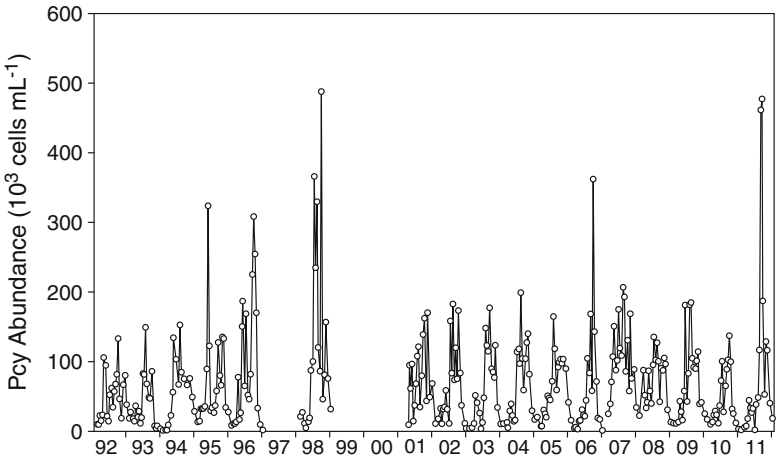

Fig. 8.4 Long-term dynamics of Pcy abundance in Lake Maggiore, Northern Italy

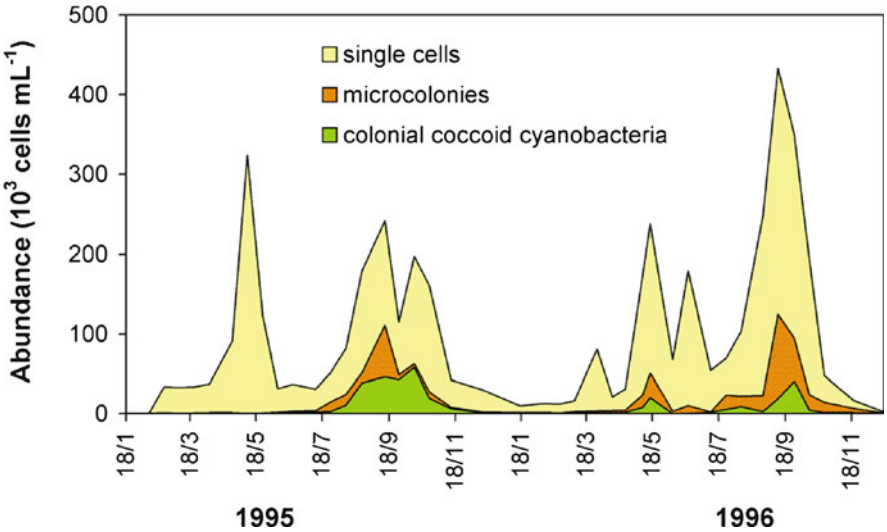

Fig. 8.5 Seasonal changes of single-cell picocyanobacteria, microcolonies and colonial coccoid cyanobacteria (mainly *Aphanothece clathrata, A. cf. floccosa* and *Microcystis* sp.) abundance in Lake Maggiore (from Passoni and Callieri 2000, modified)

than in temperate lakes in summer – 8×10^6 cells mL^{-1} (Vincent 2000). In the Antarctic Peninsula in Lake Boeckella (Izaguirre et al. 2003) Pcy abundance was as high as 3.6×10^5 cells mL^{-1} and represented up to 80% of phytoplankton biomass (Allende and Izaguirre 2003). However very low Pcy concentrations (10^2–10^3 cells mL^{-1}) were recorded in a set of shallow lakes and ponds in the Byers peninsula of maritime Antarctica (Toro et al. 2007). In contrast to all these, tropical lakes show high cell numbers (10^5–10^6 cell mL^{-1}) throughout the season with higher early-spring peaks (Peštová et al. 2008) or summer peaks (Malinsky-Rushansky et al. 1995), depending upon the interactions of Pcy with other phytoplankton.

In Lake Maggiore the pronounced late summer peak of Pcy is composed by different morphotypes, including microcolonies (Passoni and Callieri 2000) (Fig. 8.5). Microcolonies are generally present throughout the euphotic zone, albeit in low abundance in all oligotrophic lakes, e.g. representing around 25% of the single-cell forms in Lake Maggiore (Passoni and Callieri 2000). The peak abundance of Pcy microcolonies appears in summer or autumn in a variety of freshwater systems (Fahnenstiel and Carrick 1992; Klut and Stockner 1991; Komárková 2002; Szelag-Wasielewska 2003; Crosbie et al. 2003c; Mózes et al. 2006; Ivanikova et al. 2007). Such a variety of morphotypes reflects a genotypic diversity among Pcy communities that accounts for the different Pcy patterns of morphotype composition observed in spring and summer assemblages (Callieri et al. 2007; Callieri et al. 2012: Fig. 8.6). Using RT-qPCR, a type of rapid succession of individual clades of Pcy illustrates the patchy structure of the Pcy community over quite small spatial/temporal scales (Sánchez-Baracaldo et al. 2008). At the same time the co-existence of genetically and physiologically diverse *Synechococcus* spp. found in the pelagic zone of Lake Constance indicates the possible niche partitioning exploited by the different strains (Postius and Ernst 1999; Ernst et al. 2003). In Lake Maggiore the vertical partitioning of Pcy OTUs down the water column was more evident

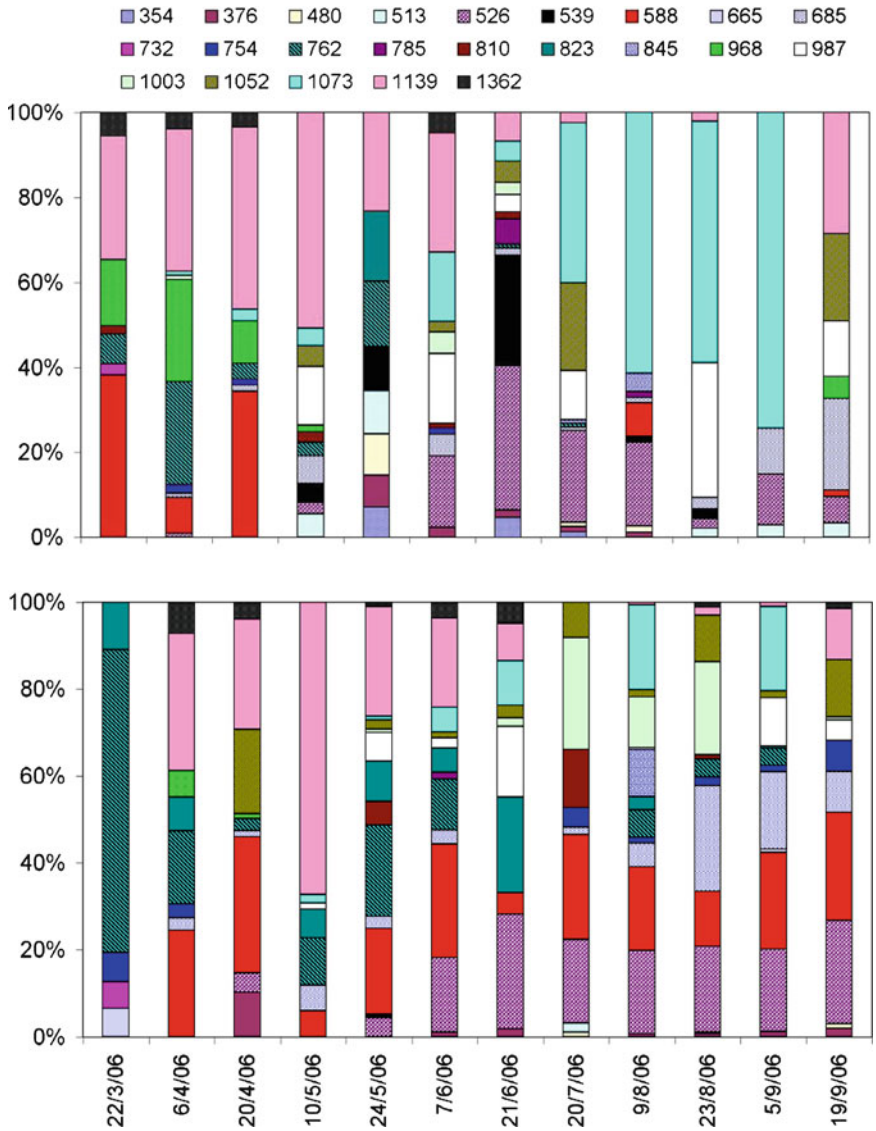

Fig. 8.6 Changes in relative percentage of the Operational Taxonomic Units (*OTU*) of Pcy in Lake Maggiore at 3 m (upper panel) and 20 m (lower panel) obtained with ARISA on the ITS-1 (Callieri et al. 2012)

during summer stratification (Callieri et al. 2012). In marine systems distinct Pcy lineages have also been shown to partition between waters having different environmental characteristics; a feature evident over large spatial scales (Fuller et al. 2006; Zwirglmaier et al. 2007, 2008). In the Sargasso Sea the community composition of Pcy varied during the season with the highest numbers of *Synechococcus* in spring and *Prochlorococcus* in summer and autumn (DuRand et al. 2001). We suggest that the new perspective of habitat-related distribution pattern of *Synechococcus* proposed for Lake Constance (Becker et al. 2007) and Lake Maggiore (Callieri et al. 2012) could be generalized to other aquatic systems as well.

Moreover, there is strong evidence that Pcy of the cyanobacterial evolutionary lineage VI *sensu* Honda et al. (1999) are not exclusively pelagic, but can also inhabit periphytic biofilm in the euphotic zone of temperate lakes (Becker et al. 2004). We should integrate our knowledge of Pcy diversity in pelagic and littoral zone habitats to better explain the dominance of certain genotypes in the water column, because the adaptability of these microorganisms may well be the key feature that accounts for their ubiquity (Becker et al. 2004).

Studies of the vertical distribution of populations of Pcy have provided important information about their response to changing physical and biological variables within the euphotic zone. Though Pcy cells are small and their settling rate negligible, their abundance and distribution within the water column can change rapidly with different thermal and light regimes, and to the presence of predators

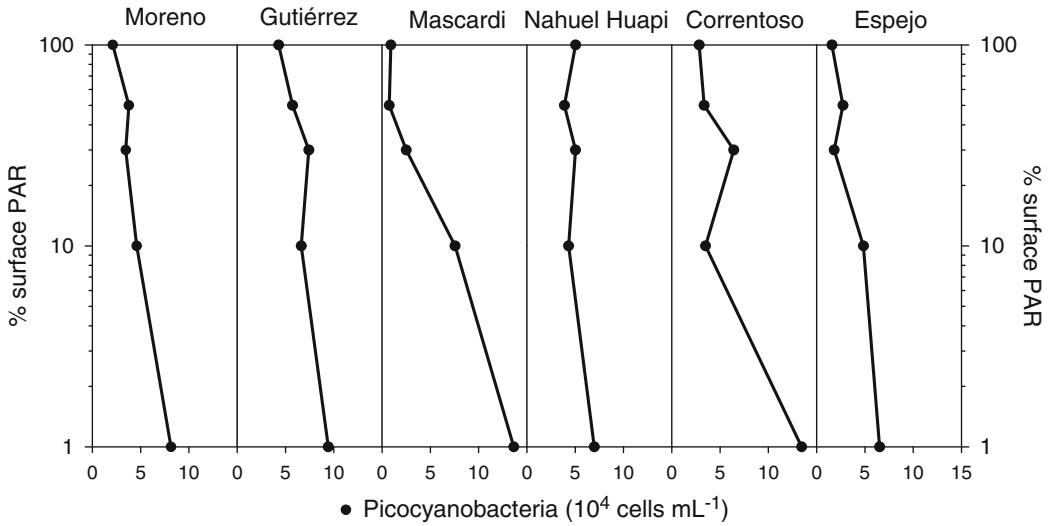

Fig. 8.7 Vertical distribution of Pcy cells in the water column of six ultra-oligotrophic lakes of northern Patagonia, Argentina (Callieri et al. 2007, modified) *PAR*: Photosynthetically Active Radiation

or viruses (Pernthaler et al. 1996; Mühling et al. 2005). Water column depth, which is often inversely related to the trophic state of the lake, is an important indicator of the presence of Pcy and/or of its abundance relative to larger species of phytoplankton. Deep, clear oligotrophic lakes typically support Pcy comprising mainly PE-rich cells; conversely in shallow, turbid lakes PC-rich cells prevail (Callieri and Stockner 2002). This disparity in the distribution of PE- and PC-rich cells is due to their characteristic spectral signature (Everroad and Wood 2006), which has been associated with particular underwater light quality (Hauschild et al. 1991; Vörös et al. 1998). In North Patagonian, in which blue light dominate the underwater light climate, Pérez et al. (2002) found that PE-rich cells typically dominate the Pcy forming a deep chlorophyll maxima (DCM) at the base of the euphotic zone (Callieri et al. 2007; Fig. 8.7). In Lake Baikal at offshore stations the Pcy are mainly PE-rich cells, whereas PC-rich cells are found at near-shore stations (Katano et al. 2005, 2008), suggesting the occurrence of water quality differences in various zones of the lake. Similar situations have been described for Lake Balaton where in the eastern basin PE-rich cells dominate, while in the western basin PC-rich cells are dominant (Mózes et al. 2006). It is the establishment of a pronounced thermocline at depth which likely favours the development of DCM, largely made up of Pcy which are suited both to low nutrient and light conditions (Modenutti and Balseiro 2002; Gervais et al. 1997; Camacho et al. 2003; Callieri et al. 2007). In Lake Tahoe Pcy dominated in the nutrient deficient upper water column during the stratified season, in a distinct vertical niche with respect to picoeukaryotes which peaked at the DCM (Winder 2009), similar to patterns found in the Oceans (Vázquez-Domínguez et al. 2008). The dynamics of DCM formed by Pcy is quite unstable and its duration is unpredictable, depending upon the strength of hydrodynamic field and biotic interactions. A good example is provided by Pcy communities in Lake Maggiore where DCM can appear, and also suddenly disappear, in just a few days (Fig. 8.8). Further, it has been shown that the interaction of different biotic and abiotic factors within the water column can affect Pcy vertical distribution patterns, with peaks of abundance in the lower metalimnion and upper hypolimnion of Lakes Huron and Michigan (Fahnenstiel and Carrick 1992); in Lake Stechlin (Padisák et al. 1998, 2003); in the metalimnion, beneath the steepest part of the thermocline, in Lake Constance and Lake Maggiore (Weisse and Schweizer 1991; Callieri and Pinolini 1995); in the metalimnion of Lake Baikal (Nagata et al. 1994); and in the epilimnion of Lake Biwa (Maeda et al. 1992), Lake Kinneret (Malinsky-Rushansky et al. 1995) and Lake Alchichica, Mexico (Peštová et al. 2008).

8.3.2 Colony Dynamics

Although colonial picocyanobacteria (CPcy) are common in meso- to eutrophic lakes, there are few publications about their life-history and ecology. CPcy are "metaphyton" that often appear in the euphotic zone as plankton, but their origins are associated with the littoral and deeper benthos communities. They occur in most cyanobacterial blooms

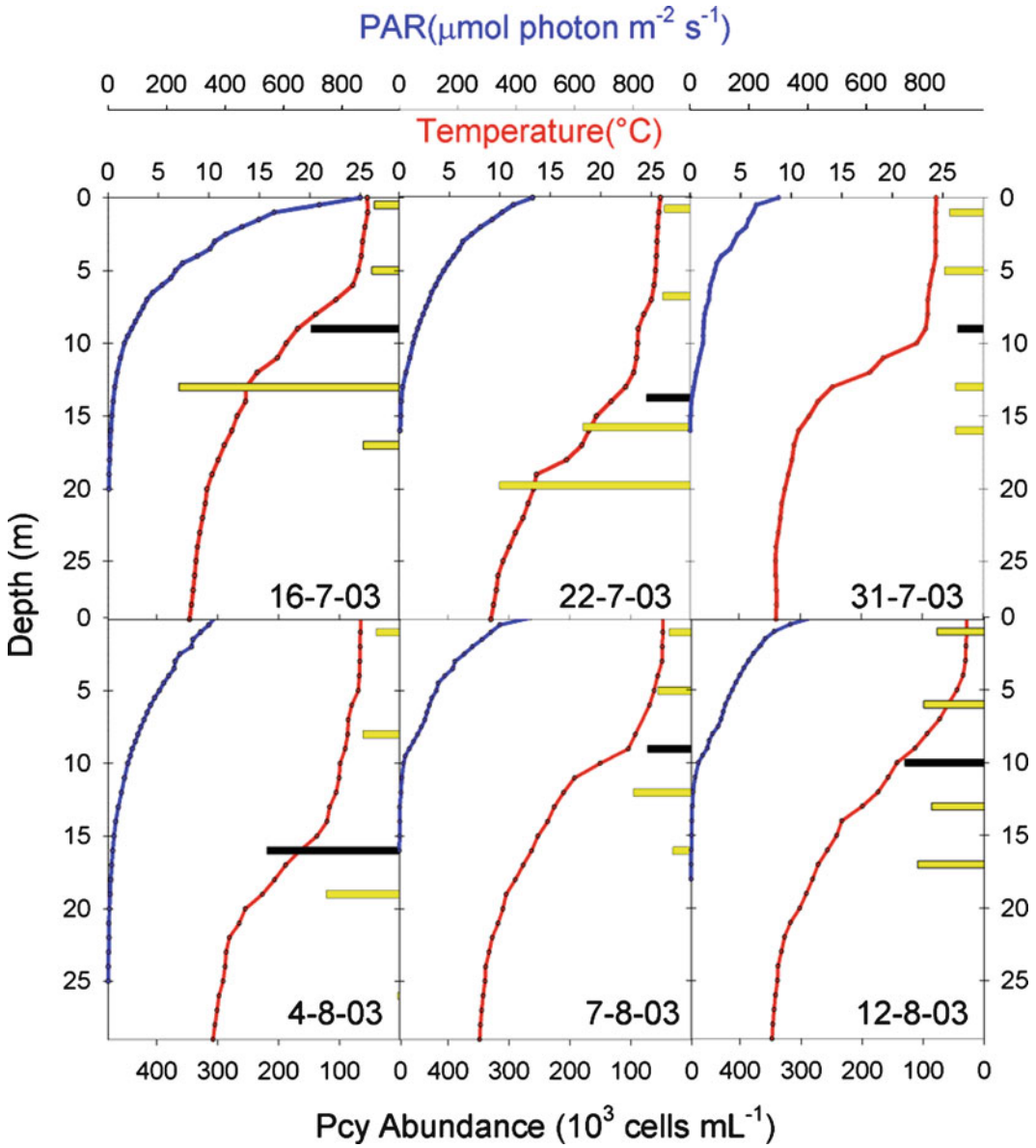

Fig. 8.8 Six vertical profiles of Pcy abundance, temperature and PAR in Lake Maggiore, during 30 days of summer stratification (C. Callieri and L. Oboti unpublished data). PAR shown in *blue* and temperature in *red line*; Pcy abundance shown with histograms: the *black* ones indicate the Pcy abundance at the depth of thermocline and the *yellow* ones the abundance at the other depths

together with larger and more common cyanobacteria like *Microcystis, Aphanizomenon* and *Anabaena*. In most cases the CPcy only constitute a small percentage of the total colonial cyanobacteria biomass of the plankton; nevertheless CPcy species alone can also form heavy blooms (Cronberg 1999).

In Sweden monitoring programs for lake water quality started in the 1970s and in some cases continues today with a focus on phytoplankton and water chemistry. A classic case is Lake Trummen, in central south Sweden that was very eutrophic, but was restored to a lower production status in 1970 by dredging and removal of 0.5 m nutrient-rich sediment (Björk et al. 1972). After dredging lake water quality consistently improved and the cyanobacterial blooms diminished both in size and duration. In spring 1975 Lake Trummen was invaded by a large number of small bream and roach from nearby Lake Växjösjön and in consequence the predation of fish on zooplankton was extremely heavy. All cladocerans disappeared and a dense water bloom of the CPcy *Cyanodictyon imperfectum* (now called *Cyanocatena imperfectum*, Fig. 8.9), appeared reaching peak densities in September that constituted 95% of the total cyanobacterial bloom. The summer of 1975 was very warm and dry creating optimal conditions that likely contributed to the heavy CPcy

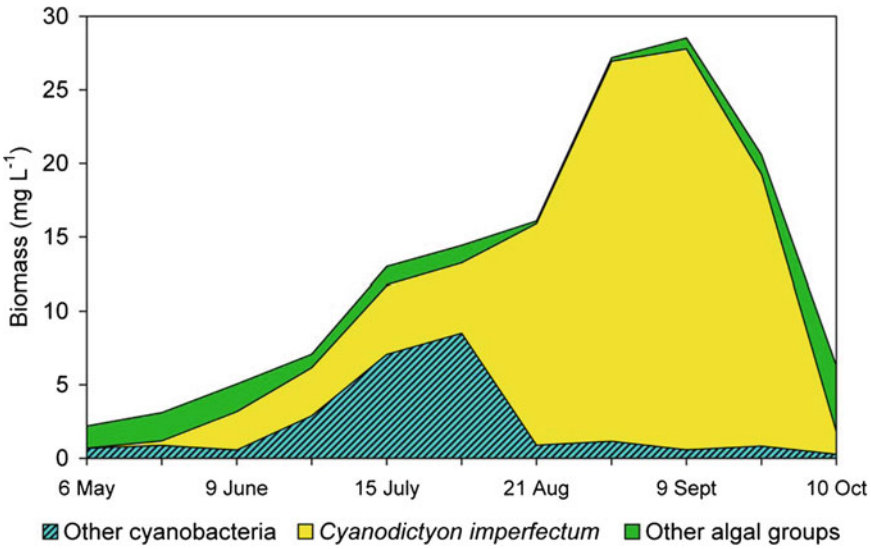

Fig. 8.9 Cyanobacterial bloom of CPcy in Lake Trummen in 1975 caused by arrival of small bream, roach and perch. The fish had reduced all cladocerans, leading to grazing by zooplankton being very reduced. *Cyanodictyon imperfectum* (= *Cyanocatena imperfecta*) dominated the bloom

bloom (Cronberg and Weibull 1981), some photos of which are shown in Fig. 8.10.

Lake Ringsjön, situated in central Scania, Sweden, consists of three basins and is one of the largest lakes in the region. The three basins have been monitored since 1982 and CPcy frequently occur in varying population densities (Fig. 8.11). In Lake Ringsjön dense algal blooms have been observed during the summer, and this lake, like Lake Trummen, also had a large population of small bream, roach and perch. During 2005–2009 Lake Ringsjön western basin was biomanipulated and small cyprinid fish were removed, which resulted in a slight reduction of phytoplankton biomass (Bergman et al. 1999). However, the CPcy increased in the beginning of the period suggesting that fish reduction in some way affected CPcy production. The cyanobacterial community was dominated by species of the genera *Microcystis, Aphanizomenon, Anabaena* and *Woronichinia*. CPcy consisted mostly of *Aphanocapsa delicatissima, Aphanothece clathrata, A. minutissima* and *Cyanodictyon imperfectum* (Cronberg 1999).

In the late 1990s park ponds in the city of Malmö, southern Sweden, were studied owing to outbreaks of heavy cyanobacterial blooms that sometimes caused large bird kills. These cyanobacterial blooms consisted of species of *Microcystis, Aphanizomenon, Anabaena, Anabaenopsis*, and sometimes also of CPcy in high densities. In 1995 a toxic bloom of *Cyanobion bacillare* appeared in the Large Slottspark Pond in Malmö and during July and August about 650 water fowl, mainly mallard ducks, died (Fig. 8.12). In late August water was analyzed for algal toxins and showed high concentrations of microcystin, about 86 μg L^{-1}, and biopsies on several dead birds showed severe damage to the liver and kidney; injuries typical of the toxin microcystin (Cronberg, unpublished data). A few years later, in 2000, the Middle Öresund Pond in Malmö exhibited an algal bloom of another CPcy species, which was a "new" CPcy species to phycological science – *Cyanodictyon balticum* (Cronberg 2003). *C. balticum* appeared from June to October with maximal development in September (Fig. 8.13). During the bloom period, the zooplankton population was also monitored, and in August, when the zooplankton declined, the CPcy increased. As long as zooplankton were present *C. balticum* biomass was kept low, but increased substantially when zooplankton disappeared. Among zooplankters, the rotifers in particular seemed to feed preferentially on the CPcy.

C. balticum is a frequent phytoplankter in the Baltic Sea where several different CPcy species are common, and they are also common in lakes in the surrounding countries. They appear in shallow meso-, eutrophic- to hypertrophic lakes and ponds where they live as part of the metaphyton on the lake bottom; but as a result of wind and convective mixing they often become distributed in the pelagic zone and become part of the plankton community (Komárek et al. 2001). Meterological and hydrological conditions doubtless play an important role in the distribution of CPcy. The composition of the surrounding soil and ground water; the presence of anaerobic littoral sediments and attendant nutrient release; even at a slightly elevated salinity, each can either alone or collectively influence the physico-chemical conditions in littoral-benthic water, and create more optimal conditions for CPcy development.

As CPcy are sometimes major components of the pelagic phytoplankton community they are influenced not only by the available nutrients, but also by other planktonic organisms. The grazing by zooplankton and by heterotrophic flagellates may reduce the number of CPcy, and the presence of small fish may also influence the CPcy populations. Furthermore, some CPcy have the ability to produce toxins, so if CPcy colonies are disrupted during the treatment process for drinking water, potentially they can affect human health.

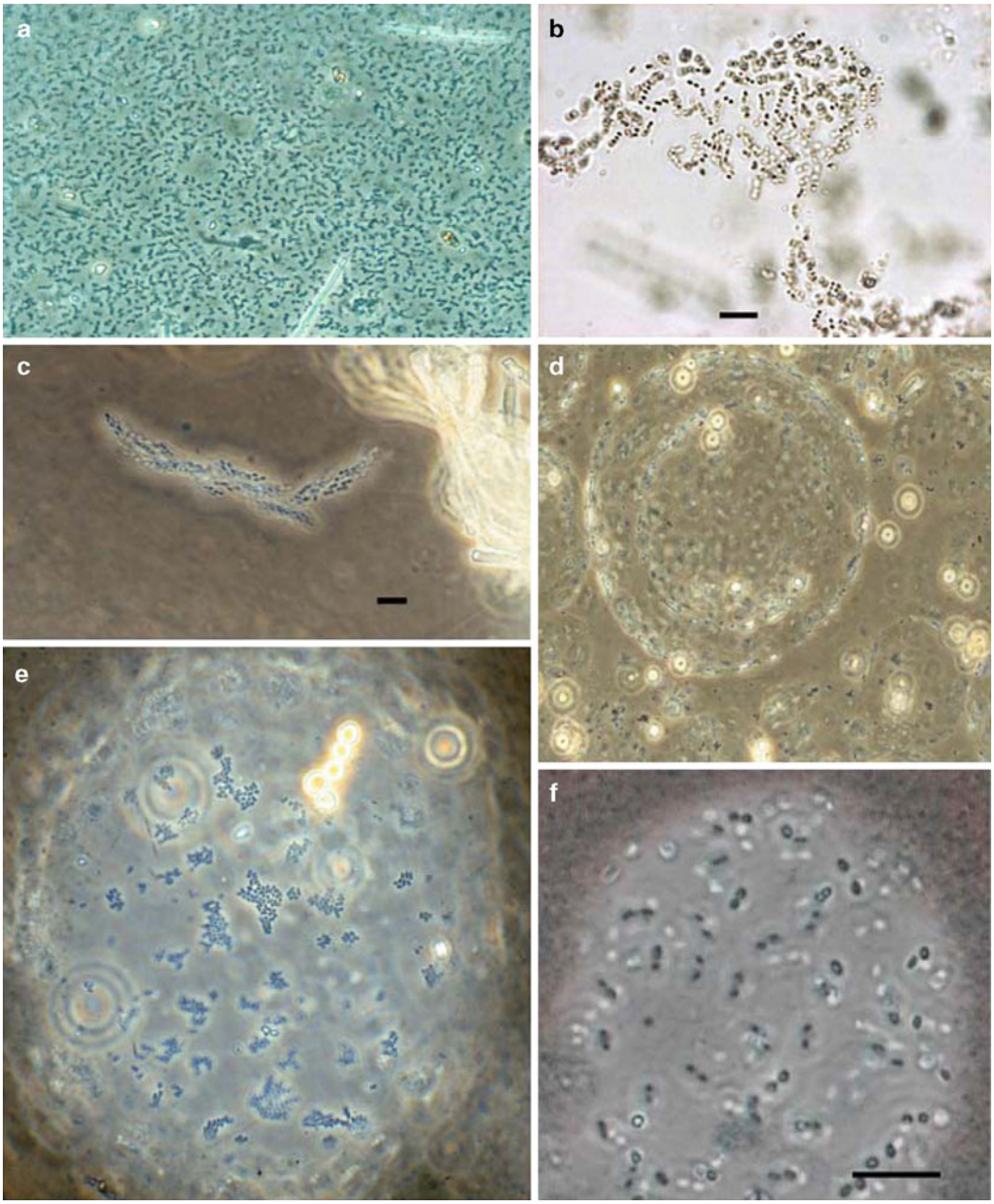

Fig. 8.10 Examples of some colonial forms of Pcy; (**a**–**b**) *Cyanodictyon imperfectum* (= *Cyanocatena imperfecta*), (**c**) *Aphanothece paralleliformis*, (**d**–**f**) *Cyanodictyon balticum* (All micrographs to the same scale: *bar* = 10 μm)

8.3.3 Single Cells Versus Microcolonies

There is a growing interest in the relationship between the status of Pcy microcolonies in a lake and the trophic state of that lake (Callieri 2010). It has been suggested that the abundance of microcolonies in temperate lakes in midsummer, when nutrients are likely to be depleted may reflect more efficient nutrient recycling, with the colonies providing a self-sustaining microcosm that offers a competitive advantage over single cells (Klut and Stockner 1991). However, Crosbie et al. (2003c) considered this hypothesis unlikely for Pcy (Crosbie et al. 2003c) in view of results for colony-forming marine alga *Phaeocystis*, where the boundary layer can strongly limit nutrient diffusion into the colonies (Ploug et al. 1999). At low phosphorus concentrations the colonial forms grow slower than the single-cell forms (Veldhuis and Admiral 1987), presumably due to the lower cell-specific nutrient fluxes in colonies (Ploug et al. 1999). But in microcolonies, formed by 5–10 cells in one plane, the duplication should not be depressed as much as in a large,

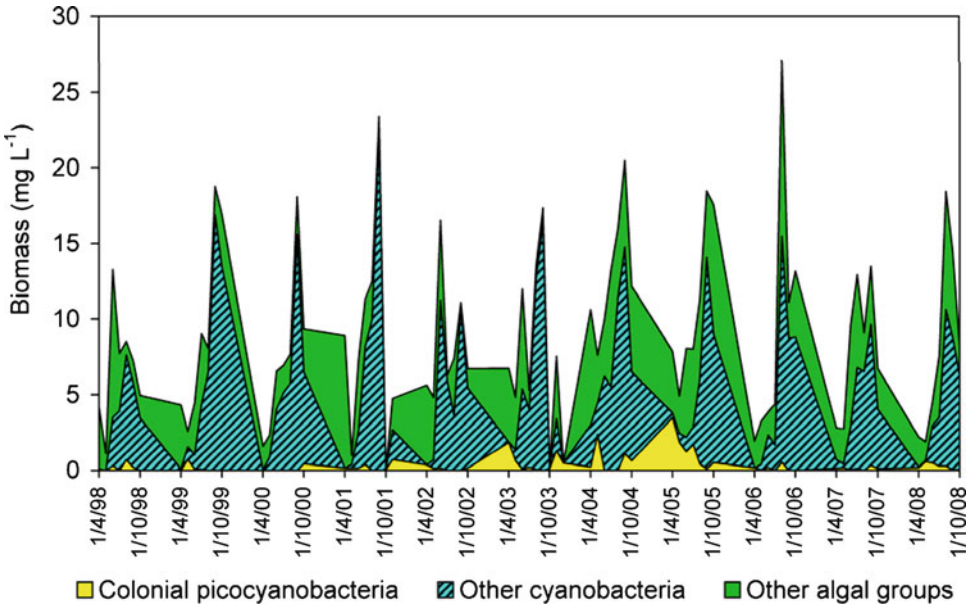

Fig. 8.11 Comparison of changes in the biomass of CPcy with those of 'other cyanobacteria' and 'other algae' in the phytoplankton of Lake Ringsjön western basin during 1998-2008. The lake was biomanipulated during 2005-2008 with 254 t small cyprinid fish being removed

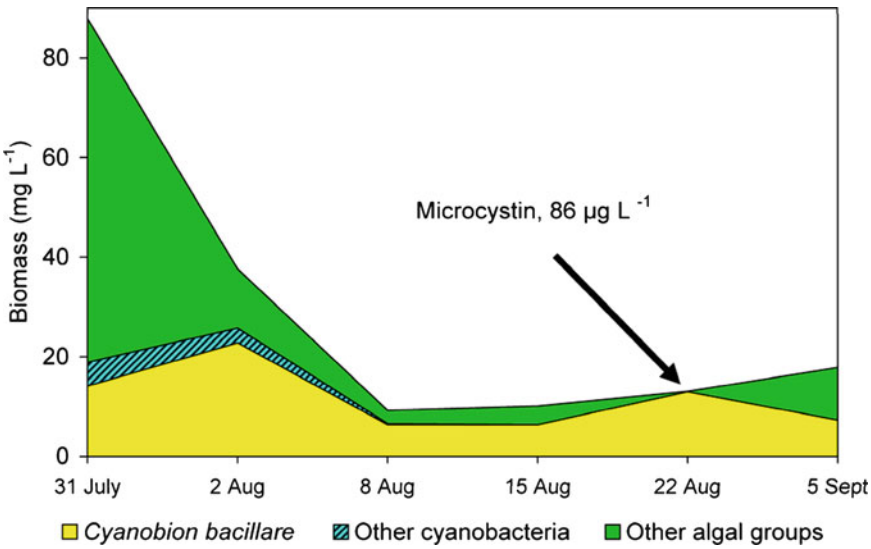

Fig. 8.12 Development of toxic *Cyanobion bacillare* in Large Slottspark Pond during the summer 1995. The black arrow indicates the periods in which microcystin was found in the lake. During the period July to September 650 water fowl died

thick colony, where the diffusion of nutrients is impeded. In this case, exudates adsorbed to the cell surface can act as rich metabolite pools (Klut and Stockner 1991). Therefore, during the initial stage of their formation a microcolony can have a selective advantage in nutrient depleted ecosystems.

Crosbie et al. (2003c) observed an increase of microcolonies in nutrient-poor surface waters in Lake Mondsee and attributed their formation to the production of photosynthate-rich mucilage in Pcy single-cells that were actively photosynthesising organic carbon. As the leakage or excretion of photosynthate has been considered one protection mechanism against photochemical damage (Wood and van Valen 1990), it is likely to also consider the effect of irradiance (PAR and UVR), at near-surface depths, as a stressor promoting clumping of daughter cells during duplication (Fig. 8.14).

To better understand genus-specific microcolony formation one must consider the factors influencing cell aggregation, despite the many differences between microcolonies and aggregates. The results by Koblížek et al. (2000) suggest that in culture *Synechococcus elongatus* aggregates rapidly if exposed to blue light (30 min, 1,000 µmol photon m^{-2} s^{-1}) owing to the effect of electron transfer downstream of PSI, with reactive oxygen radicals (ROS) probably triggering the aggregation. PSI therefore may have an important role

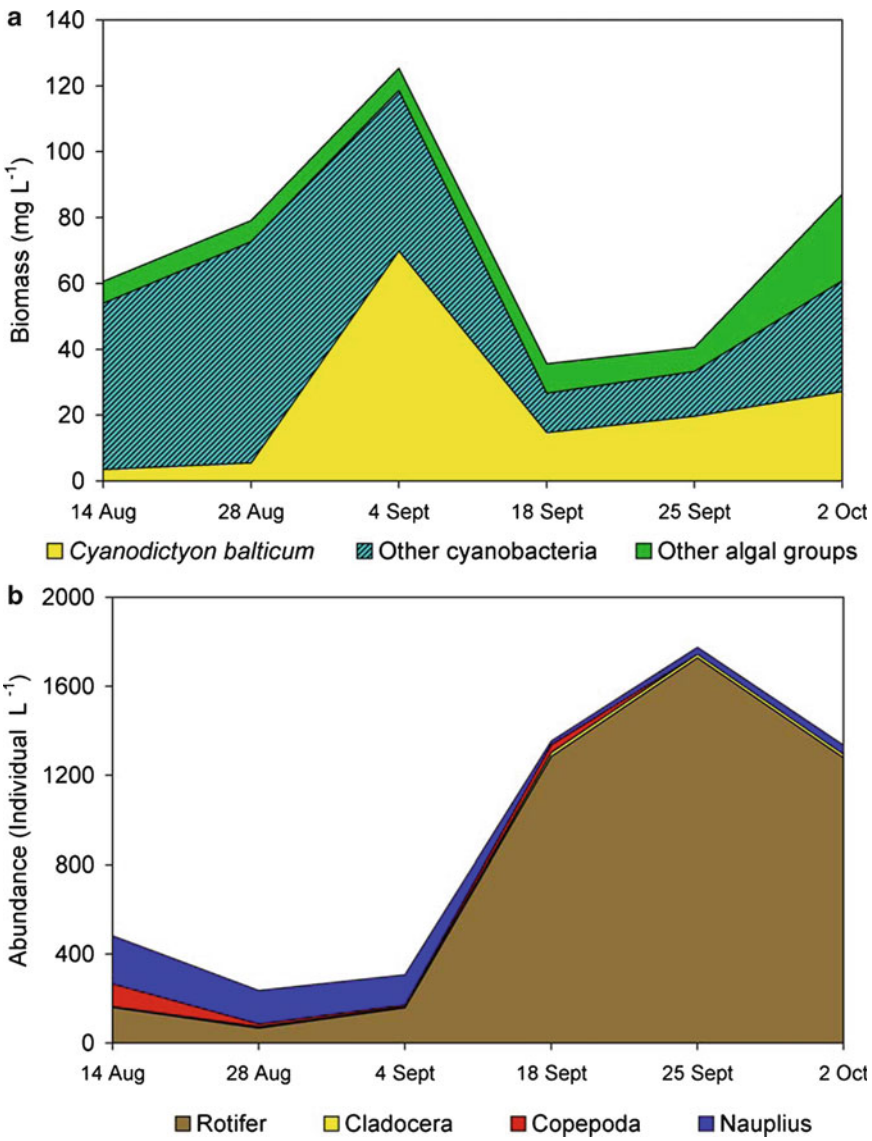

Fig. 8.13 Cyanobacterial bloom of CPcy (*Cyanodictyon balticum* and other cyanobacteria) (**a**), and development of zooplankton (**b**), in the Middle Öresund Pond during 2000

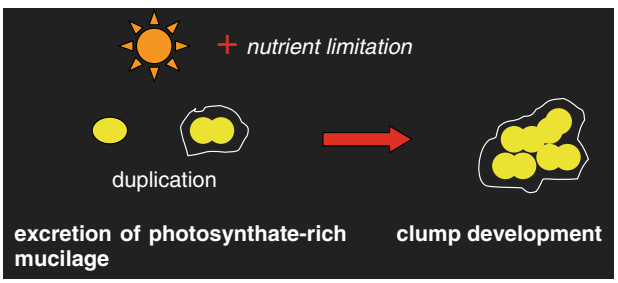

Fig. 8.14 Suggested dynamics of microcolony formation, indicating how high irradiance and nutrient deficiency could promote excretion of carbon-rich photosynthate that may in turn promote clump development due to production of daughter cells

in the first stages of microcolony formation in lakes. The influence of moderate UVR on microcolony formation was tested on PE- and PC-rich *Synechococcus* cultures (Callieri et al. 2011). Previous acclimation to low or moderate PAR influences the strain response, mainly due to carotenoid content in the cell. In general microcolony formation appears as a defence strategy of the low acclimated culture (Callieri et al. 2011).

As well as cell metabolism alterations caused by external factors such as light, other important structural changes of Pcy single-cells must be mentioned as a causative factor inducing microcolony formation. Aggregation is an ATP-independent process without any *de novo* protein synthesis

(Koblížek et al. 2000), and this indicates that some structures responsible for the aggregation must be present on the cell surface before irradiation. For example, in selected strains with different genotypes isolated from Lake Constance; Ernst et al. (1996) found that they possess a surface S-layer composed of regularly ordered globular protein layers that would facilitate colony formation. Also, in grazing (by *Ochromonas* sp.) induced microcolonies of PC-rich *Cyanobium* sp., rigid tubes from 100 nm to 1 μm long (spinae) have been observed on the cell surface (Jezberová and Komárková 2007). To what extent the formation of microcolonies is due to the presence of specific *Synechococcus* or *Cyanobium* genotypes or is the result of a specific survival strategy (Ernst et al. 1999; Passoni and Callieri 2000) needs further clarification. Unicellular *Microcystis aeruginosa* Kützing, when exposed to *Ochromonas* sp. grazing, increased the diameter of the colonies and the extracellular polysaccharides (EPS) content (Yang and Kong 2012).

A fascinating hypothesis on microcolony formation is related to the observation by Postius and Böger (1998) that exo-polysaccharides exudated by Pcy as microzones for diazotrophic bacteria growth may affect microcolony formation. This finding opens new perspectives for the study of consortial, synergistic interactions that may be of critical importance to our understanding of colony formation in Pcy.

8.3.4 Growth Rate and Occurrence Along the Trophic Gradient

The processes of cell growth and division in all the photosynthetic organisms are as tightly coupled as photosynthesis and growth rate, and are light dependent (Kana and Glibert 1987a, b; Chisholm et al. 1986). In Pcy there is little difference between marine and freshwater strains of *Synechococcus* in both cell division and growth, with cell division reaching a maximum in the afternoon, triggering an increase in cell number that proceeds in the dark (Chisholm et al. 1986; Callieri et al. 1996b; Jacquet et al. 2001). These light/dark cycles produce rhythmic cell divisions that are related to growth rate and photosynthetic activity and are driven by prevailing light conditions. Experimental laboratory evidence of the influence of light on growth rate is well documented by Fahnenstiel et al. (1991) for freshwater *Synechococcus* strains and by Campbell and Carpenter (1986a) for marine strains. These investigators have measured growth rates at light intensities up to 75 and 120 μmol photon m^{-2} s^{-1} that simulate natural irradiance levels. Kana and Glibert (1987b) have extended the light intensity limit up to 2,000 μmol m^{-2} s^{-1}, demonstrating that growth rate becomes light saturated at 200 μmol m^{-2} s^{-1}; however *Synechococcus* has a mechanism of photo-adaptation which permits cell growth and photosynthetic activity to continue at higher irradiances. Moser et al. (2009) have shown that growth rates of three Pcy strains of different pigment composition (PE-rich and PC-rich strains) and phylogenetic positions differed widely in response to light intensity and photo-acclimation (Fig. 8.15). Their results show that freshwater Pcy possess the ability of photo-adaptation, but the extent of photo-adaptation depends on the duration of photo-acclimation, and is strain-specific.

Other environmental conditions such as nutrient concentration and temperature can affect cell specific growth rates. Growth rates of pico-phytoplankton in lakes along a trophic gradient ranged from 0.10 to 2.14 day^{-1} (Weisse 1993). In the oceans the *in situ* growth rate of *Synechococcus* was likely 0.7 day^{-1} (1 doublings/day) (Furnas and Crosbie 1999). Estimates of Pcy growth rates from Lakes Biwa and Baikal were 0.65 and 0.4 day^{-1}, respectively (Nagata et al. 1994, 1996) and from Lake Kinneret ranged from 0.29 to 0.60 day^{-1} (Malinsky-Rushansky et al. 1995) all fall within published ranges. The maximum net growth rate of unicellular cyanobacteria in oligotrophic Lake Stechlin was 0.23 day^{-1} (Padisák et al. 1997) while in Lake Balaton it was 2.27 day^{-1} (Mastala et al. 1996). Based on studies of 48 lakes in Quebec, Ontario and New York State, Lavallée and Pick (2002) obtained a maximum growth rate of 1.93 day^{-1}. Pcy growth in Lake Maggiore lies between 0.28 and 1.14 day^{-1} as net growth rate and 0.91–2.36 day^{-1} as potential growth rate measured using the frequency of dividing cells (FDC) method (Callieri et al. 1996 b).

One of the selective advantages of small cell size in low nutrient environments is that cells are less limited by molecular diffusion of nutrients because of their increase in surface area-to-volume ratio (Raven 1986; Chisholm 1992). The prokaryotic structure of the Pcy cell also gives them the lowest costs for maintenance metabolism, and this has been cited as the primary reason for their success in oligotrophic conditions (Weisse 1993).

The model outlined by Stockner (1991b) of an increase of picophytoplankton abundance and biomass and decrease of its relative importance with the increase of phosphorus concentration in lakes has been widely accepted and confirmed in marine and freshwater systems (Bell and Kalff 2001). Using the extensive freshwater database of Vörös et al. (1998), Callieri and Stockner (2002), and successively Callieri et al. (2007) enriched the dataset at the ultra-oligotrophic extreme, and confirmed a positive correlation between the numbers of Pcy and extant trophic conditions in a wide range of waterbodies. Moreover, the percent contribution of Pcy to the total phytoplankton biomass and production decreased with increasing lake trophic state.

Figure 8.16 provides a comparison of the results of Callieri et al. (2007) with those of Bell and Kalff (2001). The data for Pcy from ultra-oligotrophic pristine lakes in northern Patagonia (Argentina) cluster in the same position as those

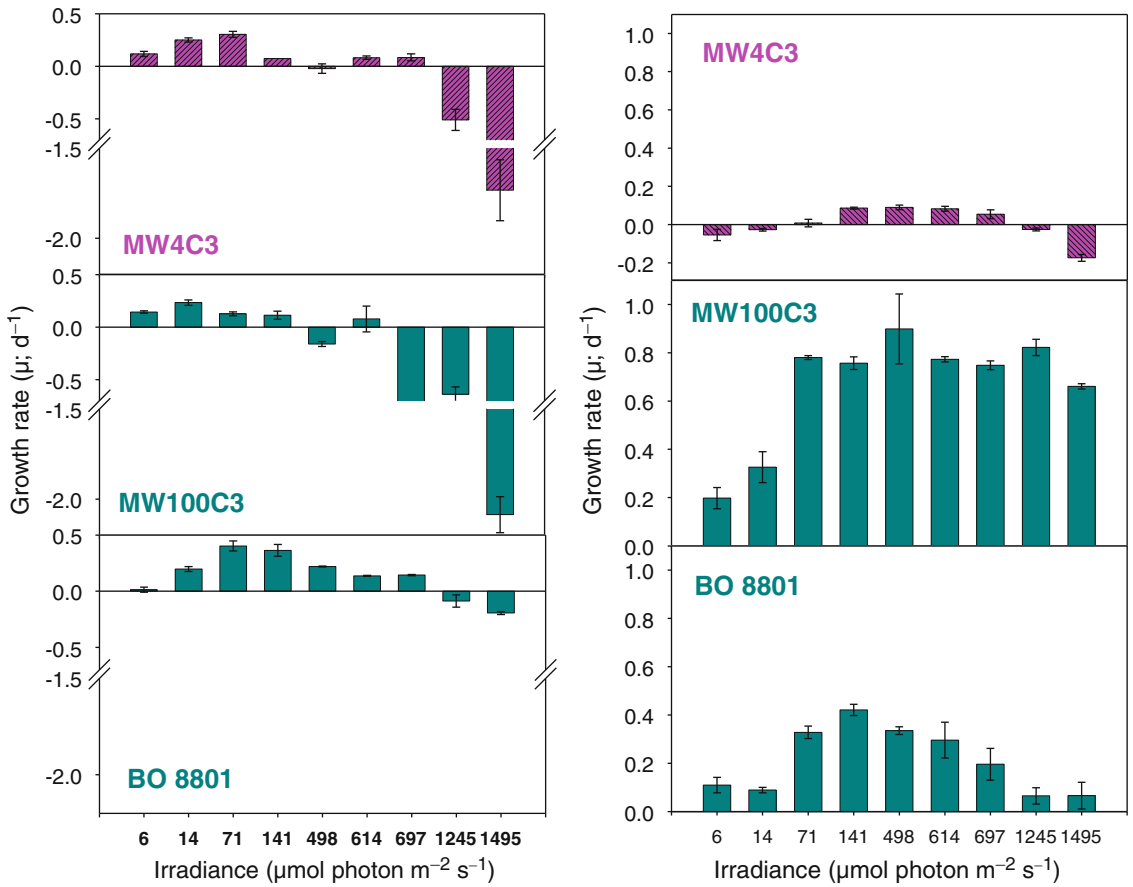

Fig. 8.15 Growth rates of picocyanobacterial strains at 9 different irradiances after 2 months acclimation to 10 μmol photon m^{-2} s^{-1} (*left*) and 100 μmol photon m^{-2} s^{-1} (*right*). MW4C3 = PE-strain Group B; MW100C3 = PC-strain Group I; BO8801 = PC-strain Group A *Cyanobium*. The growth response at high light of the two PC-strains acclimated at 100 μmol photon m^{-2} s^{-1} shows the importance of ribotype cluster membership (From Moser et al. 2009)

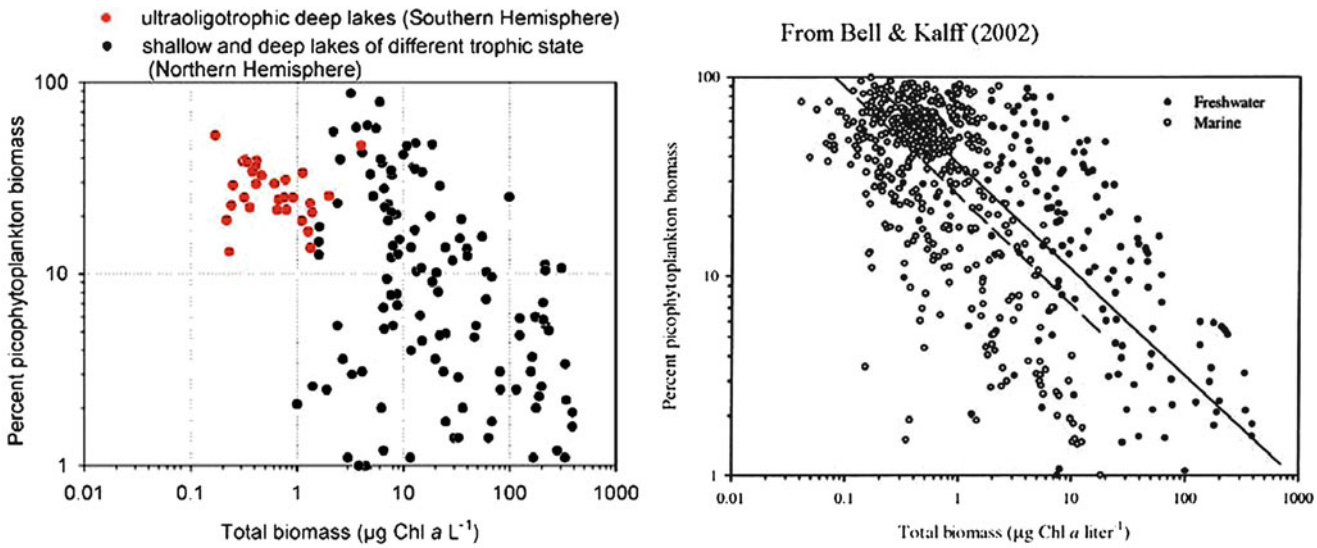

Fig. 8.16 Comparison of the results by Callieri et al. (2007) (*left*) and Bell and Kalff (2001) on % contribution of Pcy in ultraoligotrophic pristine lakes (*red circles*) and in marine systems (*open circles*)

from marine systems, suggesting a common ecological response in the various environments, despite the phylogenetic differences of the organisms.

8.4 Factors Affecting Community Dynamics and Composition

8.4.1 Biotic Versus Abiotic Regulation

Debates about the relative importance of biotic regulation versus abiotic forcing in driving population fluctuations have been recurring over decades in all fields of ecology, and microbial ecology is no exception. In a study on different terrestrial and aquatic communities Houlahan et al. (2007) found that abiotic factors provide the primary forcing that drives temporal variability in species abundance. We know that the complex changeability of community structure is related to a spectrum of environmental variability through the interplay of intrinsic (basin morphometry, thermal stratification, wind mixing) and external factors (e.g. supply of nutrients) (Harris 1980). The exploitation of environmental variability by the Pcy community is the result of evolutionary mechanistic adaptation and the interrelation with other primary producers of larger size and with predators. Adaptation to a changing environment, with phasing of fluctuating events, can subject the community to dominance by the fittest and most adaptive available species. These concepts should be evaluated on the light of the new conceptual framework of community ecology, the meta-community, which considers the communities as shaped at different spatial scales (local and regional) (Wilson 1992; Leibold et al. 2004). Therefore in the debate on biotic versus abiotic regulation of community structure and dynamics we need to consider that local communities are not isolated but are linked by dispersal of multiple, potentially interactive, species (Logue and Lindström 2008).

8.4.2 Lake Morphometry and Thermal Regime

In order to interpret Pcy dynamics in freshwaters it is imperative to take into consideration the morphometric characteristics and thermal regime of a lake. The community composition of the Pcy can strongly depend on lake typology and morphogenesis. In a survey covering 45 lakes and ponds, Camacho et al. (2003) found that picocyanobacterial development was favoured by the stability of the vertical structure of the lake; that is by the inertial resistance to complete mixing owing to vertical density differences and to a long hydrological retention time. In lakes with relatively high water inflow and short retention time, Pcy are scarce. Far from this situation are deep lakes with a complex basin morphometry such as

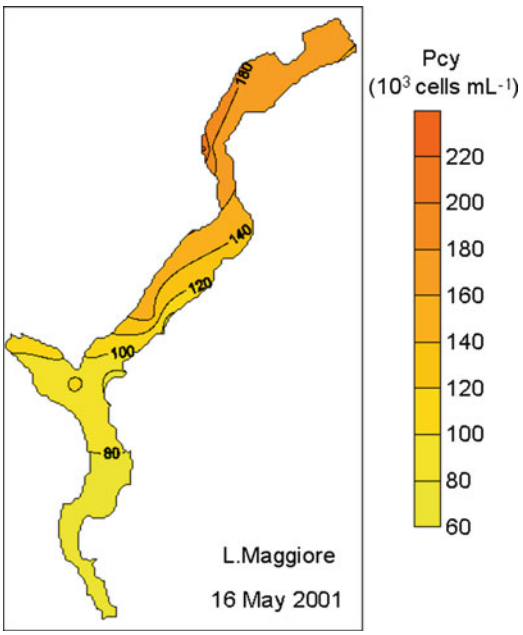

Fig. 8.17 Map of the spatial heterogeneity of picocyanobacteria (Pcy) abundance in Lake Maggiore during spring. The northern basin of the lake contributes less total nitrogen and total phosphorus than the basin for the rest of the lake (Bertoni et al. 2004, modified)

large sub-alpine lakes. In one of these lakes (Lake Maggiore) the Pcy population densities during summer stratification are high but with a pronounced North–south gradient due to a high retention time and peculiar characteristics along the lake axis (Fig. 8.17) (Bertoni et al. 2004). Lake Constance, with a different basin morphometry, has a less pronounced Pcy gradient (Weisse and Kenter 1991).

Pcy composition and abundance vary conspicuously among shallow lakes, with a strong dependence on trophic condition (Stockner 1991a; Søndergaard 1991), altitude (Straškrabová et al. 1999b and cited references), oxidization-reduction conditions (Camacho et al. 2003) and the presence of dissolved humics (Drakare et al. 2003). It is therefore difficult to predict Pcy abundance in shallow lakes without considering the physical and chemical characteristics of the water. In a study of shallow humic lakes of the Boreal Forest Zone, Jasser and Arvola (2003) found Pcy to be light and temperature limited, whereas in humic Swedish lakes dissolved organic carbon (DOC) concentration was the factor most influencing Pcy composition (Drakare et al. 2003).

Lake thermal structure influences Pcy abundance and dynamics of Pcy due to both the effect of temperature *per se* and mass movements of the water in response to density gradients. In general a temperature increase enhances the potential growth rate of phytoplankton, increasing the reaction rate of RUBISCO (Beardall and Raven 2004). Marine *Synechococcus* reacts promptly to the temperature increase in laboratory experiments (Fu et al. 2007), and in a 5-year study on Lake Balaton Pcy abundance was positively correlated

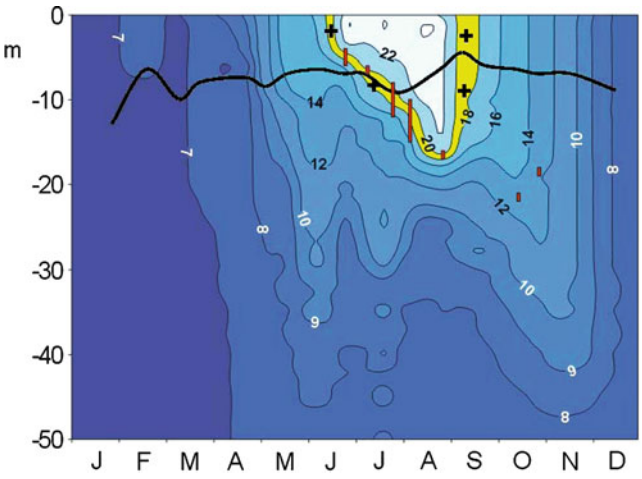

Fig. 8.18 Isotherm map of Lake Maggiore (Northern Italy), 0–50 m layer during 1998. The crosses indicate the highest values of picocyanobacteria abundance. Depths with 10 % of surface solar radiation are also given (*thick line*). Vertical bars indicate thermocline extension (From Callieri 2008)

to water temperature (Vörös et al. 2009). Nevertheless the influence of temperature on the abundance of Pcy in lakes is difficult to separate from the influence of seasonal and geographical location. The widespread assumption that temperature is the driving force for growth and development of microorganisms does not apply so clearly for Pcy in nature. Among diverse marine habitats Li (1998) found a direct relationship between Pcy mean annual abundance and temperatures below 14°C; above 14°C nitrate concentrations were very low and may therefore replace temperature as the most significant. At higher temperatures other factors can also become dominant and control Pcy growth. Weisse (1993) suggested the importance of temperature as triggering the onset of Pcy growth in marine and freshwaters, but not for regulating their population dynamics. In Lake Maggiore the maximum Pcy concentration occurs near the thermocline and at temperatures between 18°C and 20°C (Callieri and Piscia 2002) (Fig. 8.18). This provides an example of where temperature not only has a direct effect on the cell, but also helps to maintain a density gradient which hinders sedimentation (Callieri 2008). Vertical gradients of water density have a profound effect on the distribution and diversity of Pcy in the metalimnion, upper hypolimnion and mixolimnion.

8.4.3 Nutrients, Light Limitation and pH

The main difference between marine and freshwater Pcy is in the differential regulation of primary production by nitrogen, iron and phosphorus. In lakes, primarily phosphorus has been regarded as the limiting nutrient (Schindler 1977, 2006), whereas in the oligotrophic oceans nitrogen and iron are considered the ultimate nutrients limiting primary production (Tyrrell 1999). Nevertheless, in the Mediterranean Sea and in the North Pacific subtropical gyre, a climate-related shift from an N- to P-limited ecosystem over the past several decades has been observed (Moore et al. 2005), due to increased nitrogen fixation (Karl et al. 1997). Conversely, in ultra-oligotrophic lakes nitrogen deficiency, even more than phosphorus, can be the cause of the low productivity (Stockner et al. 2005; Diaz et al. 2007). Furthermore nutrient co-limitation can occur in oligotrophic systems (Mills et al. 2004), where more than one nutrient may effectively co-limit biomass production (Mackey et al. 2009). Thus, past assumptions about whether the N or P is the proximate or ultimate nutrient limiting the productivity of phytoplankton populations in marine and freshwater systems are re-opened to debate.

As regard Pcy we may infer from the Stockner model that as lakes or oceans become more nutrient depleted, i.e. oligotrophic, then the greater the importance and relative contribution of Pcy to total autotrophic biomass (Bell and Kalff 2001). The success of *Synechococcus* spp. in oligotrophic systems can also be explained by their high affinity for orthophosphate (Moutin et al. 2002) and their maximum cell specific P-uptake rates that are competitively superior to algae and other bacteria under a pulsed supply (Vadstein 2000). Actually it has been demonstrated that growth rates of marine Pcy, under limitation by NH_4^+, PO_4^{3-}, Fe or light, are seldom completely stopped; moreover, cell quotas are low as can be expected for such small cells (Timmermans et al. 2005). Iron's limited bioavailability makes it limiting despite its abundance. The siderophores are iron-chelating compounds produced by cyanobacteria (Murphy et al. 1976; Hopkinson and Morel 2009). Siderophore production can provide a competitive advantage to cyanobacteria over other algae during iron stress, and can alter the bioavailabity of iron to the aquatic community (Wilhelm 1995). Nevertheless it has been found that in diluted aqueous environment endogenous siderophore uptake is inefficient. In this type of environment, reductive Fe uptake is an effective strategy in the acquisition of organically bound iron (Kranzler et al. 2011). In laboratory studies *Prochlorococcus* and *Synechococcus* marine strains, under P-limited conditions, showed high C:P and N:P ratios thus producing new biomass with a bioelemental stoichiometry well in excess of the canonical Redfield ratio (Bertilsson et al. 2003; Heldal et al. 2003). These results allow us to envisage the potentially great importance of Pcy for enhancing carbon sequestration with the ensuing potential to change the structure and complexity of pelagic food webs (Bertilsson et al. 2003).

An alternative explanation for the relative success of Pcy to grow at low inorganic P concentrations is given by the ability of their cells to utilise, in addition to PO_4^{3-}, organic sources of phosphate. Under orthophosphate limitation, algae hydrolyse ambient organic phosphates using extracellular phosphatases and transport the orthophosphate into their cells

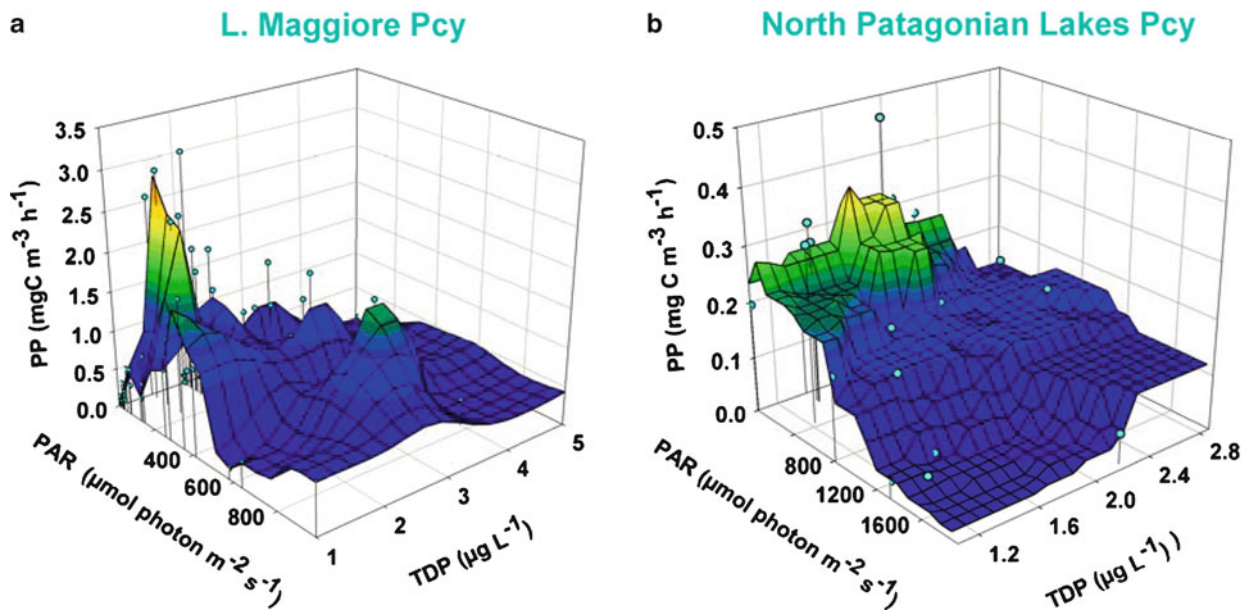

Fig. 8.19 Multiple linear regression analysis of the Pcy primary production (*PP*) versus irradiance (*PAR*) and P (total dissolved PO_4-P) in: (**a**) Lake Maggiore; (**b**) six north Patagonian lakes (Partly from Callieri et al. 2007, modified)

(Jansson et al. 1988). Whitton et al. (1991) compared the growth of cyanobacterial strains in the presence of organic or inorganic phosphate, finding a similar growth rate. The extracellular phosphatase activity (APA) in several phytoplankton species has been demonstrated by the enzyme-labelled fluorescence (ELF) technique (Nedoma et al. 2003; Štrojsová et al. 2003). This technique permits both the quantification of the enzyme produced and the microscopic localization of the enzyme. Pcy can produce alkaline phosphatases under conditions of phosphate starvation (Simon 1987) but, up to now, none has been observed to show APA-activity using the ELF technique (A. Štrojsová, 2002 personal communication). The method detects only phosphomonoesterase, not phosphodiesterase, activity; therefore caution is needed in interpreting the significance of surface phosphatase activity (Whitton et al. 2005). A genetic study on marine strains has revealed inter-strain variability in the presence and/or absence of genes governing P-acquisition, scavenging and regulation (Moore et al. 2005). Such genetic variability will clearly influence the different physiological responses to low P concentrations of individual strains, e.g. the production of APA.

There are other alternative ways for Pcy to overcome P limitation. Two pathways are of interest: one has been discovered from the presence of genes necessary for phosphonate utilization in the genome of Pcy (Palenik et al. 2003; Ilikchyan et al. 2009). Phosphonates are refractory high-molecular-weight components of the dissolved organic phosphorus (DOP) pool. Marine and freshwater Pcy have the *phn*D gene, which is thought to encode a phosphonate-binding protein, and in a study induction of *phn*D expression in P-deficient media was demonstrated (Ilikchyan et al. 2009). This suggests that in P-limiting conditions *Synechococcus* is able to survive utilizing this refractory form of DOP, derived also from a common herbicide. The other alternative derives from the ability of marine cyanobacteria, and particularly *Prochlorococcus*, to substitute sulphate (SO_4^{2-}) for PO_4^{3-} in lipids, thus minimising their phosphorus requirement by using a 'sulphur-for-phosphorus' strategy. The strategy of synthesizing a lipid that contains sulphur and sugar instead of phosphate could represent a fundamental biochemical adaptation of Pcy to dominate severely phosphorus-deficient environments (Van Mooy et al. 2006, 2009).

There is evidence that ammonium is the preferred form of nitrogen for *Synechococcus* in culture (Glibert and Ray 1990), but when ammonium is exhausted *Synechococcus* can take up nitrate, thanks to a regulatory mechanism that can induce expression of nitrate reductases (Bird and Wyman 2003). Also, under severe N-limitation Pcy can alternatively use the nitrogen reserve that exists in phycobiliproteins as amino acids storage molecules (Grossman et al. 1993). The success of Pcy under low light conditions is tightly coupled with competition for limiting nutrients. In this way, low-light adaptation in *Synechococcus* is probably of greatest ecological advantage when low-P conditions constrain the growth of all autotrophs (Wehr 1993). Thus the pulsed addition of P can have an interactive effect, because it increases the prevalence of larger algae that can alter the light climate, thereby increasing light limitation which will enhance the growth of Pcy.

Good evidence on the interplay between P, irradiance and primary production of Pcy and how it is mediated in the field is difficult to envisage, but some clues come from the comparison of six ultra-oligotrophic North Patagonian lakes and from the subalpine Lake Maggiore (Fig. 8.19) (Callieri et al. 2007). In the ultra-oligotrophic lakes Pcy production was

inversely significantly correlated to PAR but not to P, indicating that in such extremely nutrient deplete ecosystems, low P concentrations were not the limiting resource driving Pcy production. One interpretation of these results is that high irradiance is photo-inhibiting Pcy production and hence is the key driving variable and not phosphorus concentration. Conversely, in the oligo-mesotrophic Lake Maggiore neither P nor light were not correlated to Pcy production. Similar finding are reported by Lavallée and Pick (2002) who found a lack of correlation between pico-phytoplankton growth rates and any form of dissolved phosphorus.

Light is an important factor in niche differentiation for Pcy. The response of Pcy to different light intensity has been studied both in laboratory experiments and *in situ*, and it has been shown that the optimum growth rate of *Synechococcus* occurs at low light intensities (Waterbury et al. 1986), notably at a quantum flux of 45 μmol photon m^{-2} s^{-1} where their highest growth has been observed (Morris and Glover 1981). These findings agree with field observations where the maximum peak abundance has been found deep in the Atlantic mixed layer (Glover et al. 1985), and in the DCM (deep chlorophyll maximum) of Lake Stechlin (Gervais et al. 1997) and of North Patagonian ultra-oligotrophic Andean lakes (Callieri et al. 2007). In lakes worldwide Pcy have been found at a variety of depths and light irradiance (Fahnenstiel and Carrick 1992; Nagata et al. 1994; Callieri and Pinolini 1995; Callieri and Piscia 2002), confirming the classification of *Synechococcus* as a euryphotic organism (Kana and Glibert 1987b). One explanation of Pcy tolerance and adaptation to high irradiance is the identification of a process that prevents photo-damage in open ocean Pcy by maintaining oxidized PSII reaction centres, channeling the electrons from PSI to oxygen through a specific oxidase (Mackey et al. 2008). Because of this process Pcy possess an efficient mechanism for dissipating PSII excitation energy, decreasing any potential photo-damage. Nevertheless the relative phylogenetic complexity of the *Synechococcus* and *Cyanobium* genera does not presently permit the simple discrimination of high light- and low light-adapted ecotypes, as has been attained for *Prochlorococcus* (Scanlan and West 2002; Ahlgren and Rocap 2006).

Synechococcus ecotypes exhibit differences in their accessory pigments that affect their adaptation to spectral light quality. It was found that in highly coloured (humic) lakes, non-phycoerythrin cells dominated numerically, while in clearer, oligotrophic hard-water lakes, phycoerythrin-rich cells were the most abundant (Pick 1991). The influence of underwater light quality on the selection of Pcy types having different pigment content has been studied in many aquatic systems, covering a wide spectrum of trophic states and underwater light quality (Vörös et al. 1998; Stomp et al. 2007). Vörös et al. (1998) found that the percentage of PE-rich cells in the total Pcy community increased with

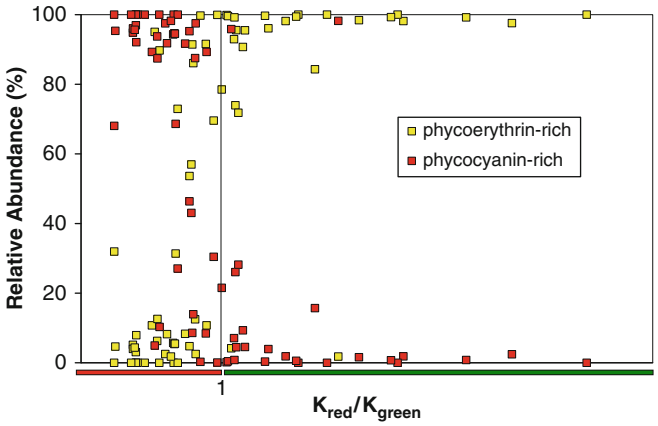

Fig. 8.20 Relative abundance of phycocyanin-rich cells (*PC*) and phycoerythrin-rich cells (*PE*) in different aquatic systems in relation to the underwater light climate expressed as the ratio between the extinction coefficient of red and green wavelengths (K_{RED}/K_{GREEN}). When the K_{RED}/K_{GREEN} ratio is >1 the extinction of *red light* is high and the dominant underwater light is *green*. Very low values of K_{RED}/K_{GREEN} ratio indicate a *red* dominant underwater radiation (Modified from Vörös et al. 1998)

increasing values of the K_{RED}/K_{GREEN} ratio, while concurrently the total chlorophyll *a* concentration decreased and the waters became more transparent and less productive (Fig. 8.20). In laboratory experiments, it has been shown that Pcy grow better when they have a phycobiliprotein whose absorption spectrum is complementary to that of the available light (Callieri 1996) and subsequent experiments showed that PE-rich cells prevail in green light and PC-rich cells in red light but when grown together in white light, can co-exist, absorbing different parts of the light spectrum (Stomp et al. 2004). The importance of red light for phycocyanin and biomass production has been shown in laboratory experiments with a PC-rich *Synechococcus* strain (Takano et al. 1995), while blue and green wavelengths of light are used more efficiently than red of similar intensity by PE-rich *Synechococcus* (Glover et al. 1985).

The pigment composition of Pcy represents a characteristic spectral signature that can define individual strains, but closely related strains can have different pigment composition (Everroad and Wood 2006). In particular both pigment types have been found in several non-marine Pcy clusters (Crosbie et al. 2003b). A new clade, sister to *Cyanobium*, was reported from oceanic waters, based upon phylogenetic analysis of concatenated 16S rDNA and *rpo*C1 data sets (Everroad and Wood 2006). This large clade includes both PE-rich and PC-rich strains. Similarly, marine cluster B (MC-B) also contains PE-rich and PC-rich strains, and this cluster is polyphyletic, consisting of at least two different sub-clusters (Chen et al. 2006). The phylogeny derived from the *cpc*BA operon of the green PC pigment was better able to separate differently pigmented Pcy than 16S rRNA-ITS phylogeny (Haverkamp et al. 2008). The ecological implication of these

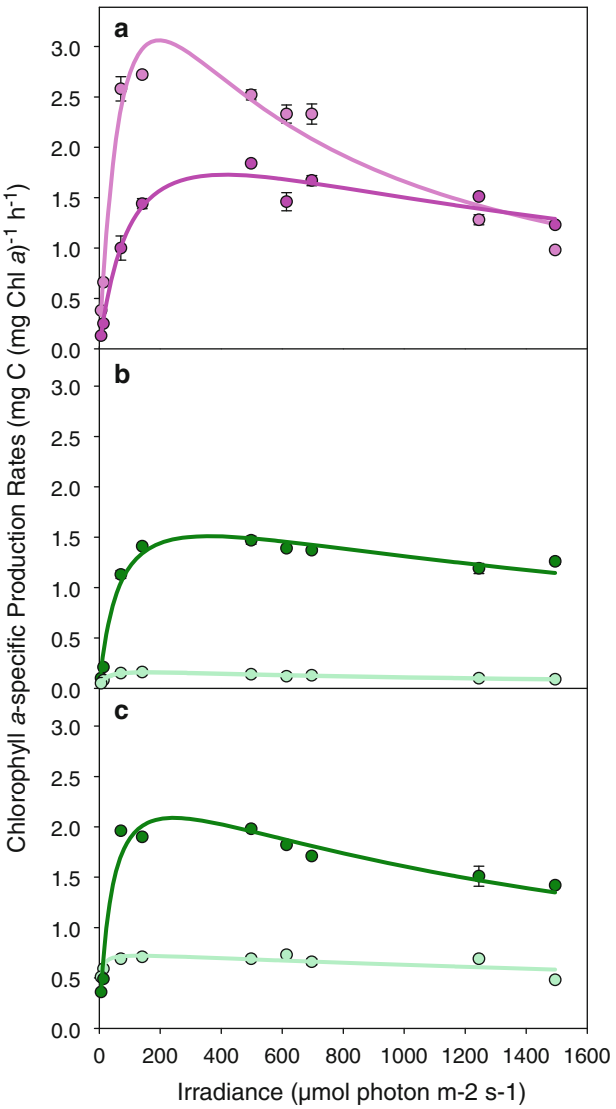

Fig. 8.21 Photosynthesis/Irradiance (P/E) *curves* of three Pcy strains: (**a**) PE-cells MW4C3 from the Group B, Subalpine cluster; (**b**) PC-cells MW100C3 from Group I; (**c**) PC-cells BO8801 from Group A, *Cyanobium* cluster. *Dark symbols* = medium light acclimation (100 µmol photon m^{-2} s^{-1}); *light symbols* = low light acclimation (10 µmol photon m^{-2} s^{-1}) (From Moser et al. 2009, modified)

findings is that *Synechococcus* from different lineages can occupy different niches; or alternatively, if the environment offers greater variability and more suitable niches, like in the Baltic Sea (Haverkamp et al. 2009) or in Lake Balaton (Mózes et al. 2006), they can coexist.

Laboratory experiments with freshwater strains from different phylogenetic groups acclimated at low and medium irradiance show that photosynthetic responses are strain-specific and sensitive to photo-acclimation (Callieri et al. 2005; Moser et al. 2009) (Fig. 8.21). PE-rich Pcy from Group B, subalpine cluster I (*sensu* Crosbie et al. 2003a, b), are more sensitive to photo-acclimation than PC-rich cells from Group I and from Group A, *Cyanobium gracile* cluster. Therefore ecophysiological differences seem to be more related to the pigment type. Nevertheless the extent of photo-adaptation is strain-specific and depends on the duration of the photo-acclimation (Moser et al. 2009).

Synechococcus strains are most often grown in a medium at a neutral pH (Stanier et al. 1971). Their preference for neutral or slightly alkaline conditions is also evident in their abundance and distribution patterns in freshwater ecosystems (Stockner 1991b). The trend towards Pcy disappearance with decreasing pH and their replacement by picoeukaryotes has been noted in three dystrophic Canadian lakes (Stockner and Shortreed 1991) and in several low pH, humic Danish lakes (Søndergaard 1991). Also in seven humic lakes situated in Lappland (Sweden) pH was among the abiotic variables most affecting pico-phytoplankton distribution and abundance (Drakare et al. 2003). The effect of lake acidification on the microbial community can be indirect by altering community structure and hence carbon flow to higher trophic levels, or direct by inducing physiological stress. In a study on a Swedish acidified lake before and after liming a non-edible CPcy, *Merismopedia tenuissima,* was the dominant in the late summer phytoplankton community in the naturally acidic lake, but the population was removed by liming (Bell and Tranvik 1993; Blomqvist 1996). Unfortunately the authors present no data on Pcy abundance in this lake. However, they suggest a likely allelopathic mechanism to explain the population dynamics of *Merismopedia tenuissima* (Blomqvist 1996; Vrede 1996). In Lake Paione Superiore, an acid sensitive lake above the tree line in the Italian Alps, Pcy populations are very low, and their contribution to microbial food webs appears to be negligible (Callieri and Bertoni 1999). In alpine lakes the effect of low pH and of photo-inhibition has been indicated as the major cause of low Pcy numbers found in those lakes (Straškrabová et al. 1999a). The presence of a shift from Pcy to net plankton has been described in mesocosm experiments (Havens and Heath 1991), and it has been noted that as pH declines the proportion of larger species tends to increase and become dominant (Schindler 1990). Nevertheless, to our knowledge no experimental studies of the influence of pH on Pcy strains have been done, so at this stage it is difficult to discuss ranges of pH tolerance by Pcy or physiological mechanisms of adaptation to low pH in lakes. The only possible generalisation at this stage is that Pcy and many CPcy are not common in lakes with a pH <6.0, and are seldom mentioned or included in studies on acidic lakes because they are probably in low abundance or absent.

8.4.4 Ultraviolet Radiation

There is considerable evidence that ultraviolet radiation (UVR) has a pronounced influence on aquatic organisms and on their community structure in both marine and freshwaters

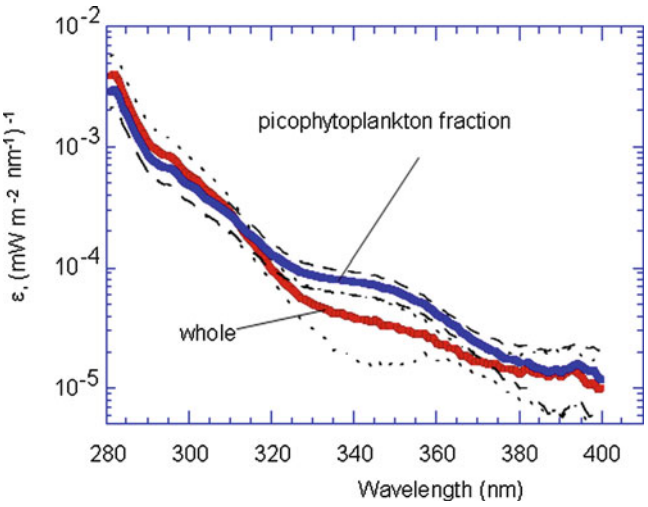

Fig. 8.22 Biological weighting functions (mW m^{-2} $nm^{-1})^{-1}$ for inhibition of photosynthesis for L. Cadagno picophytoplankton (*blue line*) and whole assemblage (*red line*) on 13 September 1999. *Broken lines* show estimated 95% confidence interval for individual coefficient estimates (From Callieri et al. 2001)

(Häder et al. 2007). Picoplankton are thought to be particularly vulnerable to UVR because: (1) their small size does not permit the intracellular production of sunscreen compounds (Garcia-Pichel 1994); (2) the small 'package' effect leads to higher pigment-specific absorption (Morel and Bricaud 1981) and (3) the distance between the cell surface and the nucleus (DNA) is shortened and the DNA damage induced by UV-B is increased. Thymine dimers like cyclobutane pyrimidine dimer (CPD) are frequently built upon DNA lesions under UV-B radiation and have been recovered in marine phytoplankton (Buma et al. 1995) and Argentinean lakes (Helbling et al. 2006).

Although the higher vulnerability of picoplankton is predictable in theory, contrasting results have been obtained in field studies. Laurion and Vincent (1998) studying size-dependent photosynthesis in a sub-arctic lake have shown that cell size is not a good index of UVR sensitivity. Further, they indicated that Pcy are less sensitive to UVR fluxes and that genetic difference between taxa, more than size, are important in determining the tolerance to UVR; while other authors obtained evidence of an higher vulnerability of smaller algae to UVR (Kasai et al. 2001; Van Donk et al. 2001). A high Pcy sensitivity to UVR radiation in comparison to nano-phytoplankton was observed in the biological weighting functions (BWF) in a high altitude alpine lake by Callieri et al. (2001) (Fig. 8.22). A possible interpretation of these contrasting results is that small cells are likely more susceptible to DNA damage than large cells but they are able to acclimate faster, within hours (Helbling et al. 2001), and are more resistant to photosystem damage (Villafañe et al. 2003).

The spectral quality of the UVR exposure, its duration and photon flux density, strongly influences the effect on phytoplankton communities (Harrison and Smith 2009). The damaging power of radiation generally increases from PAR through UV-A into the UV-B wavebands, but this general pattern may still be questioned (Harrison and Smith 2009). There is evidence that many aquatic organisms react promptly to UV-B stress by producing protective substances such as mycosporine-like amino acid compounds (MAAs) (Sinha and Häder 2008), which have absorption maxima ranging from 310 to 359 nm (Carreto et al. 1990; Karentz et al. 1991). In particular cyanobacteria react in response to UV-A radiation by producing an extracellular yellow-brown pigment – scytonemin, that absorbs most strongly in the UV-A spectral region (315–400 nm) (Garcia-Pichel and Castenholz 1991; Dillon et al. 2002). The sunscreen capacities of MAAs and scytonemin are higher if they are present concurrently, and their production is considered an adaptive strategy of photo-protection against UVR irradiance (Garcia-Pichel and Castenholz 1993). Also it is recognized that the UV-B induced production of CPD is counterbalanced by repair mechanisms based on the production of enzymes known as photolyases (Jochem 2000).

Therefore aquatic microorganisms have numerous mechanisms of protection against UVR which influence their global community responses in nature. It has been recognized that many of the effects of solar UVR are caused by wavelengths in the UV-A range, which are not affected by changes in stratospheric ozone (Sommaruga 2009). The higher photo-inhibiting effect of UV-A than UV-B on different size fractions of phytoplankton has been described in several different lakes (Callieri et al. 2001, 2007; Villafañe et al. 1999) and in marine habitat as well (Villafañe et al. 2004; Sommaruga et al. 2005). Callieri et al. (2001) explain the negligible impact of UV-B on *in situ* phytoplankton production with the lower weighted irradiance brought about by the high K_d at short wavelengths and low incident flux, whereas with UV-A the weighted irradiance is higher due to a greater incident flux and lower K_d.

Mixing is an important factor affecting the degree of exposure to UVR. Vertical mixing transports the cells to depth where active repair takes place and subsequently re-exposes them to higher UVR, upon transport again to near surface depths. Species which form surface blooms, like colonial *Microcystis aeruginosa*, can also withstand high UVR, synthesizing carotenoids and MAAs (Liu et al. 2004). Toxin biosynthesis by *M. aeruginosa* may also be influenced by UVR, with the idea that microcystins present inside or outside the cell function as a metal ligands to reduce metal toxicity (Gouvea et al. 2008). The hypothesis that UVR and the bioavailability of trace metals can act as a trigger for microcystin production is fascinating, and would help to explain the selective advantage of cystin production by

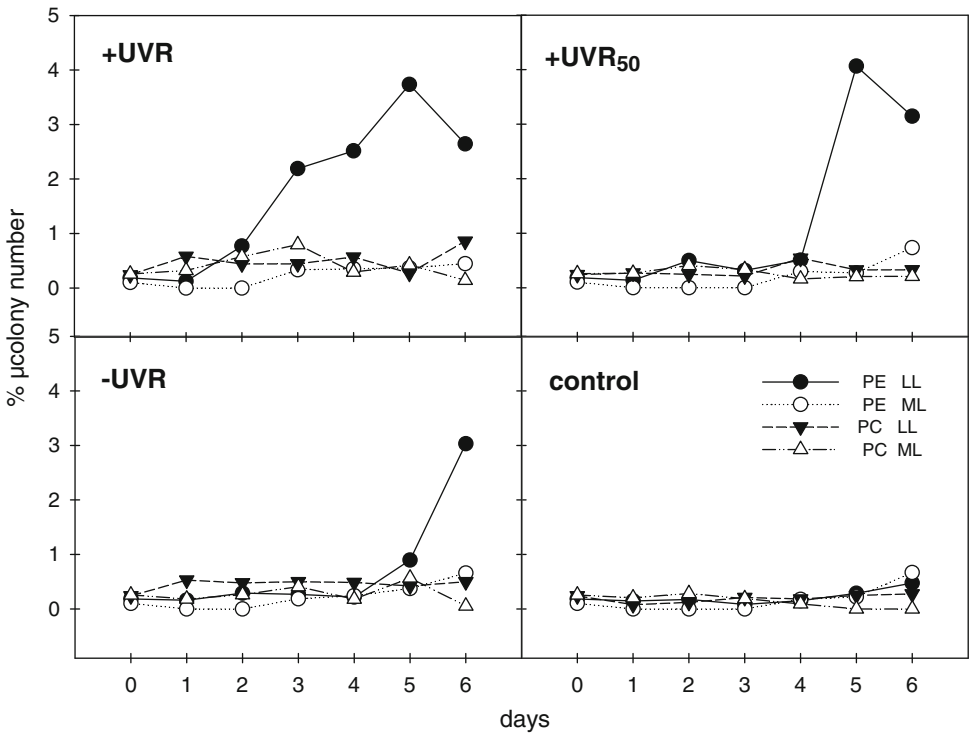

Fig. 8.23 Percentage of microcolony number on the sum of single-cell plus microcolony number. PE and PC-rich *Synechococcus* strains in the treatments (+UVR, +UVR50, -UVR, and control) at two acclimations (LL: 10 µmol m^{-2} s^{-1} and ML: 100 µmol m^{-2} s^{-1}), during experiment times (6 days) (from Callieri et al. 2011, AEM)

M. aeruginosa, but it is not yet proven. To explain the resistance of CPcy to UVR it is interesting to note that the colonial morphotype of *Microcystis* can synthesise substances such as D-galacturonic acid, which is the main component of the slime layer of *Microcystis* (Sommaruga et al. 2009), and which may hence provide a protective function. To better understand the formation of microcolonies (Callieri et al. 2011) used PE-rich and PC-rich *Synechococcus* strains of different ribotypes acclimated at moderate (ML) and low (LL) light, and exposed the strains to different levels of UVR under controlled conditions. PE-rich *Synechococcus* acclimated to LL had a low carotenoid/chlorophyll a (car/chl) ratio but responded faster to UVR treatment, producing the highest percentages of microcolonies (Fig. 8.23) and of cells in microcolonies. Conversely, the same strain acclimated to ML, with a higher car/chl ratio, did not aggregate significantly. These results suggest that microcolony formation by PE-rich *Synechococcus* is induced by UVR if carotenoid levels are low. PC-rich *Synechococcus* formed a very low percentage of microcolonies in both acclimations even with low car/chl ratio. It is likely that some *Synechococcus* strains react to UVR finding a refuge through a morphological adaptation, inclusive of slime layer protection, similar to that noted in *Microcystis* (Sommaruga et al. 2009). Therefore, in the equilibrium between single cells versus microcolonies or even larger colonial morphologies, the importance of solar radiation (UVR and PAR) should not be underestimated but considered together with other important factors like the nutrient status of the ecosystem and grazing.

8.4.5 Biotic Interactions

Grazing studies have been stimulated by several new methodologies. Rates of Pcy removal by grazers have been measured using five basic techniques: (1) metabolic inhibitors (Campbell and Carpenter 1986b); (2) diffusion chambers and the dilution technique (Landry and Hassett 1982); (3) fluorescent labelled particles (FLP) (Sherr et al. 1987); (4) direct cell counts (Waterbury et al. 1986) and (5) radioisotope-labelled prey (Iturriaga and Mitchell 1986). Some of these methods have been improved (Landry et al. 1995; Sherr and Sherr 1993) and others developed with the use of modern techniques, e.g. by combining FLP and flow cytometry for cell counting (Vázquez-Domínguez et al. 1999) or using a RNA stable isotope probing technique (Frias-Lopez et al. 2009). In the past various growth inhibitors were tested, including the eukaryote inhibitors colchicine and cycloheximide, which have been used to stop protozoan Pcy grazing activity (Campbell and Carpenter 1986b; Caron et al. 1991). Liu et al. (1995) used kanamycin as an effective growth inhibitor of *Synechococcus* and

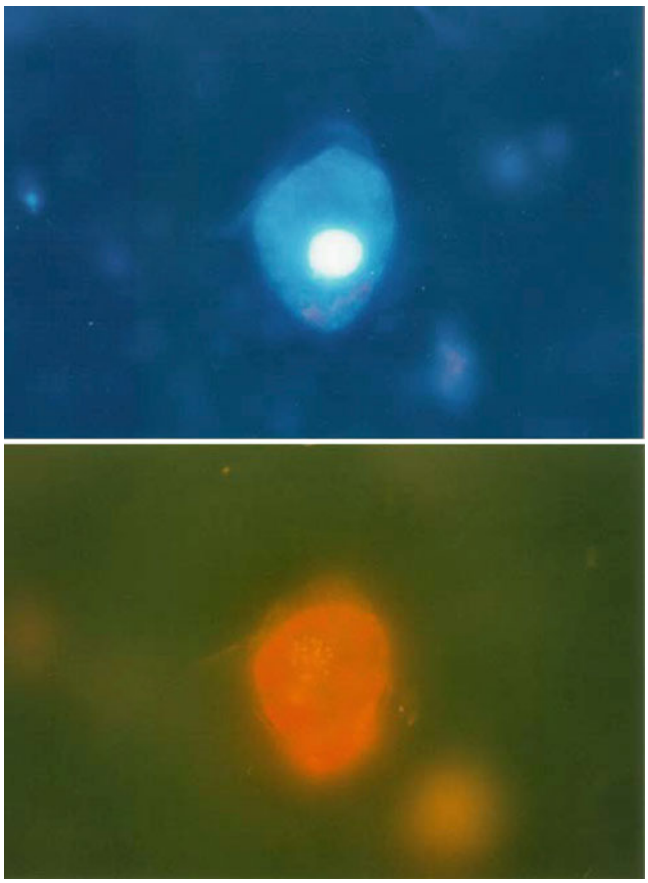

Fig. 8.24 The ciliate *Limnostrombidium* sp. coloured with DAPI and visualised under UV (*above*) and blue (*below*) excitation (epifluorescence microscope 787.5×). In DAPI the nucleus is clearly visible and in blue excitation vacuoles full of *yellow* Pcy appear

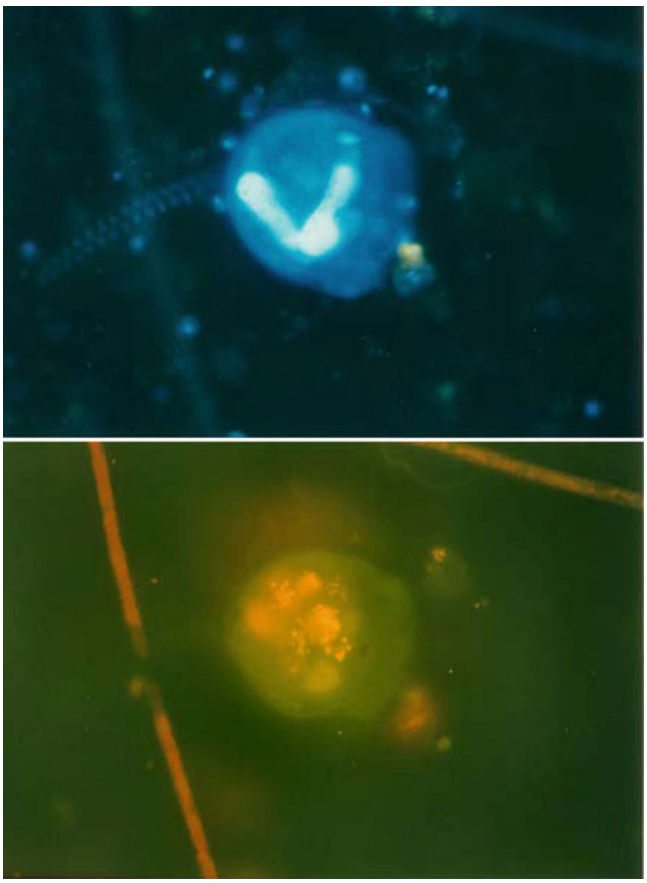

Fig. 8.25 The ciliate *Vorticella* sp. coloured with DAPI and visualized under UV (*above*) and blue (*below*) excitation (epifluorescence microscope 787.5×). In DAPI the nucleus is clearly visible and in blue excitation vacuoles full of *yellow* Pcy appear

Prochlorococcus to estimate growth and grazing rates. Using this approach the mortality of Pcy in marine systems due to grazing has been estimated to range from 43% to 87% of growth rate in marine systems (Liu et al. 1995).

It is not surprising that the existence and continuing development of so many methodologies to measure grazing has produced such diverse and often contradictory results in the literature. For example Sherr et al. (1991) estimated that in Lake Kinneret ciliate carbon requirement could not be obtained only from a Pcy energy source, and they suggest that picoeukaryotic cells must be grazed as well to fulfil growth requirements. However, Šimek et al. (1996) show that some of the most common freshwater ciliate species can survive solely on a diet of Pcy. A tentative annual balance of energy flow in a deep oligotrophic lake estimated that between 83% and 97% of the carbon produced by Pcy is taken up by protozoa and channelled to metazooplankton (Callieri et al. 2002). Nevertheless, there are large losses of organic carbon through respiration during this transfer, along the trophic chain (Sherr et al. 1987). These discrepancies and contrasting results have enhanced the discussion to improve our understanding of the impact of different grazers (protozoans and metazooplankon) on Pcy and on energy transfer, along with this "trophic repackaging".

Heterotrophic and mixotrophic nanoflagellates and small ciliates have been recognised as the most important grazers of Pcy (Stockner and Antia 1986; Bird and Kalff 1987; Weisse 1990; Christoffersen 1994; Šimek et al. 1995; Sanders et al. 2000). Among the ciliates (Fig. 8.24), oligotrich species and some scuticociliates, which are sometimes at the borderline between nano- and microplankton (<30 μm), can be important picoplanktivores in lakes (Šimek et al. 1995; Callieri et al. 2002). Šimek et al. (1996) have summarised three ecological categories of freshwater ciliates with different feeding strategies and a decreasing importance of pico-size prey in their diet. Among the most efficient suspension feeders there are some very active Pcy grazers, e.g. *Vorticella aquadulcis* (Fig. 8.25), *Halteria grandinella*, *Cyclidium* and *Strobilidium hexachinetum*. These protozoa are able to graze 560, 210, 80, 76 Pcy ciliate^{-1} h^{-1}, respectively, with clearance rates highly variable among taxa, 11–3150 nL × cells × h^{-1} (Šimek et al. 1996). In a warm-monomictic saline lake in

Mexico lower uptake rates by vorticellids and mixotrophic *Euplotes* have been measured, ranging from 16 to 227 Pcy ciliate^{-1} h^{-1} (Peštová et al. 2008), but the authors noted the importance of both groups as selective Pcy grazers. Large mixotrophic ciliates, common in ultra-oligotrophic south Andean lakes, are also recognised as preying upon Pcy (Modenutti et al. 2003; Balseiro et al. 2004).

Despite the importance of ciliate grazing on Pcy in some systems, it is generally recognized that among protozoa, both heterotrophic and mixotrophic nanoflagellates are responsible for 90% of the grazing of Pcy and bacteria; whereas ciliates accounted for only 10% (Pernthaler et al. 1996). A study on Lake Maggiore showed that heterotrophic nanoflagellates (HNF) ingested from 0.5 to 3 Pcy h^{-1}, while ciliates ingested from 18 to 80 Pcy h^{-1} (Callieri et al. 2002). Nevertheless, at the community level, the grazing impact of HNF was one order of magnitude higher than that of ciliates (maxima: 8,000 Pcy mL^{-1} h^{-1} and 400 Pcy mL^{-1} h^{-1}, respectively) (Callieri et al. 2002). In Lake Tanganika similar results were obtained with the higher impact of HNF (av: 8027 Pcy mL^{-1} h^{-1}) than of ciliates (maxima: 1355 Pcy mL^{-1} h^{-1}) on Pcy grazing in the dry season (Tarbe et al. 2011). Pernthaler et al. (1996) have emphasised the influence of community composition and taxa-specific clearance rates on the grazing impact on bacteria and Pcy. The size of the prey, its morphological characteristics and nutritional value have been indicated as important factors in the selection carried out by the predators (Šimek and Chrzanowski 1992; Jezberová and Komárková 2007; Shannon et al. 2007). In particular the involvement of the proteinaceous cell surface (S-layer) as grazing protection has also been suggested for freshwater *Actinobacteria* (Tarao et al. 2009). Morphological characteristics can therefore be considered as group-specific traits and can greatly influence the success of the group in an ecosystem (Tarao et al. 2009). Protozoa grazing and in particular nanoflagellates can influence the characteristics of bacterial and Pcy communities and lead to changes in their structural and taxonomic composition. A laboratory study with 37 *Synechococcus* strains showed clearly that prey selection discriminates at the strain-specific level (Zwirglmaier et al. 2009).

The selection of food as described for metazooplankton generally takes place during food capture and processing (Porter 1973). According to the theory of "selective digestion" prey selection takes place inside the food vacuoles (Boenigk et al. 2001). The fate of the prey is decided at the moment of digestion, with the possibility of very fast prey-excretion after the uptake. Knowledge of the mechanism of Pcy consumption and excretion/digestion is species-specific both for prey and predator. Boenigk et al. (2001) demonstrated that prey characteristics and predator satiation strongly influence the ingestion and digestion process, e.g. the digestion strategies of *Cafeteria*, *Spumella* and *Ochromonas* are different; with Pcy rapidly excreted while bacteria were directly digested in the food vacuoles. Amoebae can perform food selection in the food vacuole and excrete the toxic or unpalatable prey items similarly to nanoflagellates (Liu et al. 2006; Dillon and Parry 2009).

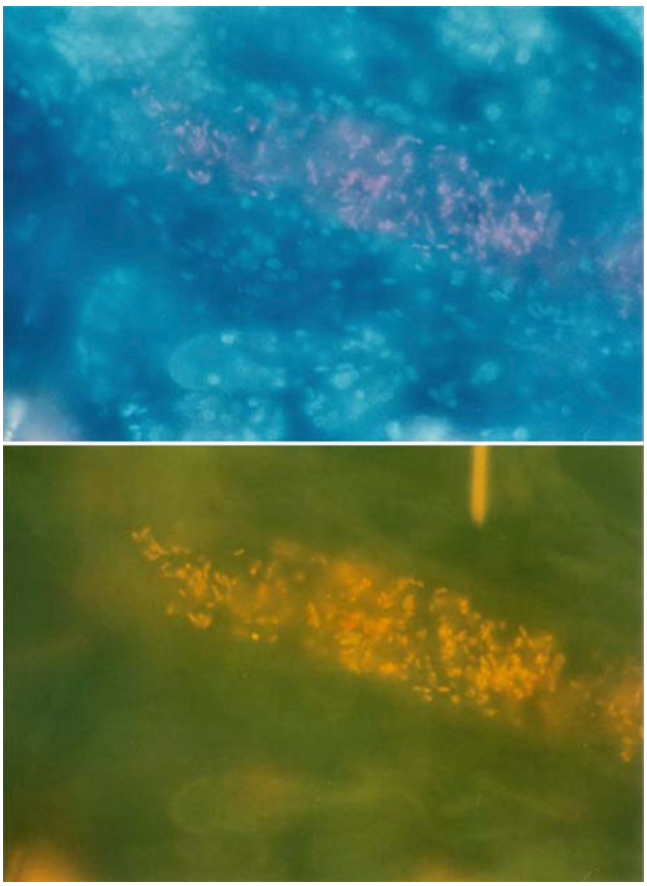

Fig. 8.26 Cladoceran gut coloured with DAPI and visualised under UV (*above*) and blue (*below*) excitation (epifluorescence microscope 787.5×); in blue excitation *yellow* Pcy appear

Ciliates and nanoflagellates can also serve as a trophic link between Pcy production and *Daphnia* production, thereby upgrading the nutritional value of Pcy as a food source by producing essential lipids such as sterols (Martin-Creuzburg et al. 2005; Martin-Creuzburg and Von Elert 2006; Bec et al. 2006). Among mesozooplankton, *Daphnia* has the capacity of feeding on a wide particle size range (1–50 µm), filtering Pcy as well (Gophen and Geller 1984; Stockner and Porter 1988) (Fig. 8.26). Together with *Daphnia*, several cladoceran genera, including *Bosmina, Eubosmina* and *Ceriodaphnia*, are able to ingest Pcy (Weisse 1993). Suspension-feeding cladocerans may have a direct grazing effect on Pcy and an indirect effect by regenerating nutrients (Carrillo et al. 1996; Balseiro et al. 1997). The recycling of excreted nutrients moves the nature of algal-bacterial interactions from one of competition to commensalism (Reche et al. 1997).

An important effect of grazing by *Daphnia* on Pcy functioning was observed in laboratory experiments (Callieri et al. 2004), where there was an increase in P and C cell-specific uptake by Pcy and in their photosynthetic efficiency. This activity could have been related to the release of P by *Daphnia*, which has been reported to be 5% of the total P-pool per day (Boersma and Wiltshire 2006). Another possible conjecture is that nutrients are replenished during the passage of Pcy through the digestive tracts of consuming daphnids (Porter 1975; Stockner 1991b). There is evidence that nutrient-limited green algae pass through the gut of *Daphnia* intact and alive (Van Donk and Hessen 1993) and that during passage they can use some of the P released in the gut (Boersma and Wiltshire 2006).

Among lake studies of Pcy, only a few refer to the impact of copepod grazing, particularly calanoid copepods. It has been shown that copepods have a stronger negative effect on ciliates than do *Daphnia* (Burns and Schallenberg 1996) and that top-down effects in the short term are stronger in oligotrophic ecosystems than in eutrophic ones (Burns and Schallenberg 2001). The mesocosm experiments of Zöllner et al. (2003) showed the structuring and cascading effects of the cladoceran *Daphnia hyalina* cf. *galeata* and copepods (50% *Eudiaptomus* spp. and 50% copepodite stages of cyclopoid copepods) on microbial food web structure. These investigators found a decrease in Pcy that was probably due to the selective feeding of copepods on intermediate-sized ciliates and a strong increase in the concentrations of HNF, as was previously found for bacteria (Jürgens and Jeppesen 2000). Copepods prey selectively and efficiently on ciliates and algae in the size range 20–40 μm (Yoshida et al. 2001), thereby triggering a trophic cascade, enabling high numbers of HNF and the potential for a greater mortality of Pcy (Zöllner et al. 2003). Sundt-Hansen et al. (2006) have shown that in marine mesocosms, copepods have a profound structuring effect on the pelagic food web, and thus directly and indirectly regulate the abundance of Pcy predators. In this way, the strength of the trophic cascade downward to Pcy depends substantially on the structure of the food web and the inventory of zooplankton species present (Gismervik 2006; Van Gremberghe et al. 2008).

Rotifers can either act directly on Pcy populations by grazing or indirectly by preying on nanoflagellates and small ciliates (Stockner and Shortreed 1989; Arndt 1993) (Fig. 8.27). Many planktonic rotifers (*Keratella cochlearis*, *K. quadrata*, *Polyarthra dolichoptera*) feed on particles in the size-range 0.5–3 μm, interspecific variation in food selection being dependent on differences in the corona sizes of the consuming species (Ronneberger 1998). As Stockner and Antia (1986) asserted, Pcy are within the size range suitable for grazing by nauplii and early copepodite stages of copepods. This possibility has been partially confirmed by the direct estimation of the grazing rate on Pcy and bacteria

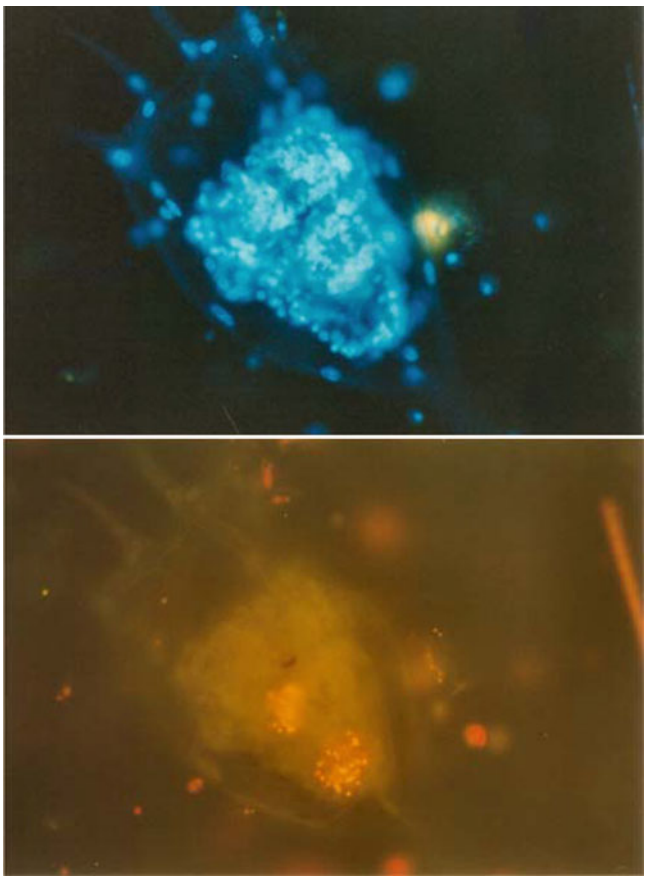

Fig. 8.27 The rotifer *Keratella cochlearis* coloured with DAPI and visualised under UV (*above*) and blue (*below*) excitation (epifluorescence microscope 787.5×); in blue excitation vacuoles full of *yellow* Pcy appear

by a copepod naupliar stage in a marine system (Roff et al. 1995). At present there are few studies, all in marine systems, on the role of bivalve and gastropod larvae as possible picoplanktivores. In one of these, Bell (1991) showed that *Crepidula aculeata* and *Littoraria scabra*, two gastropod larvae, can thrive on a Pcy and bacteria diet. Furthermore, the larvae of a bivalve, *Mercenaria mercenaria*, have been shown to ingest and grow on a *Synechococcus* strain (Gallager et al. 1994). Thus, there now appears to be some coupling between microbial and littoral benthic food webs in aquatic ecosystems.

Zaret and Suffern (1976), who studied the grazing effect of zooplankton on phytoplankton in Gatun Lake, Panama, found that CPcy were not grazed, and they suggested that this was probably due to the fact that cells were embedded in a gelatinous matrix. Blomqvist (1996) and Vrede (1996) have reported *Merismopedia tenuissima* to be resistant to grazing by the dominant grazer *Eudiaptomus* in a clear water, oligotrophic lake in central Sweden. Thus, from what little is known at this time, it seems probable that the mucilaginous colonial morphology, as in larger colonial cyanobacteria, is

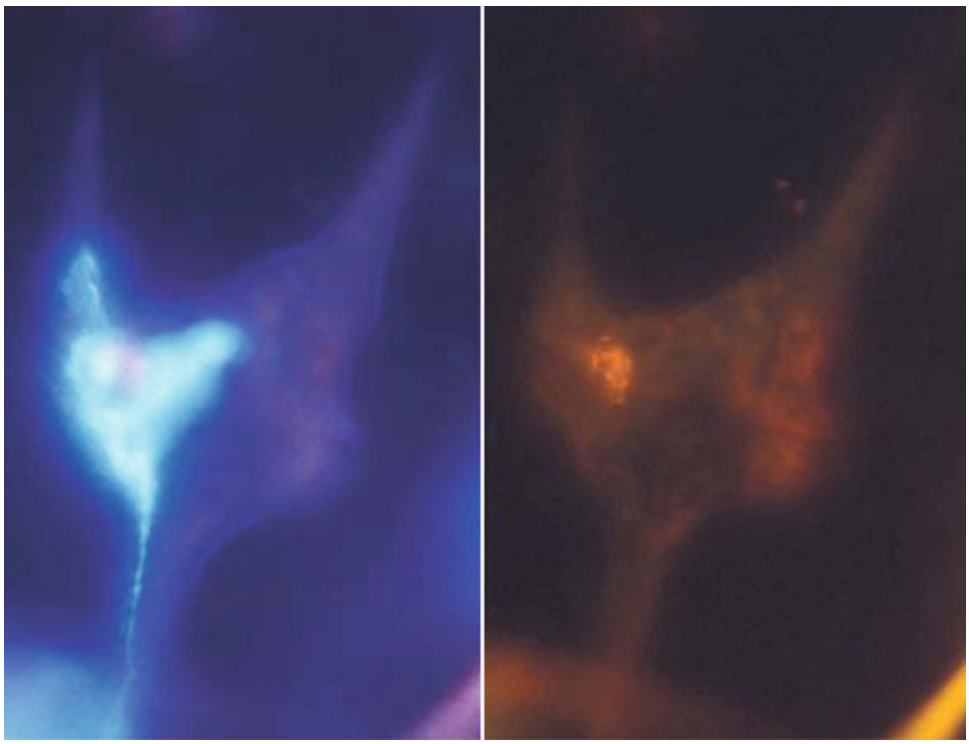

Fig. 8.28 The dinoflagellate *Ceratium hirundinella* from Lake Maggiore observed with the vacuole full of yellow Pcy. Coloured with DAPI and visualised under UV (*left*) and blue (*right*) excitation (epifluorescence microscope 787.5×)

an effective anti-grazing adaptation that, when coupled with the possibility of allelopathic strategies of CPcy colonies, creates the perfect, coupled anti-predation device for these CPcy species. This may very well be the reason for their ubiquity and abundance in lakes.

While predation has been recognised as an important top-down structural and dynamic control of Pcy, little attention has been directed towards the study of other ecological interactions such as symbiosis (Adams 2000; Chap. 23). Marine cyanobacterial symbionts (or 'cyanobionts': Taylor 1982) provide an example of proto-cooperation. A symbiotic relationship between oceanic unicellular cyanobacteria and a tintinnid, *Codonella* sp., was demonstrated by Carpenter and Foster (2002). In an especially interesting study, Foster et al. (2006) used molecular methods to amplify prokaryotic symbiont rRNA sequences from individual marine cells of various marine eukaryotes. Their results showed 53% of the cyanobacterial symbionts to be closely related to *Synechococcus* sp. and 3% to *Prochlorococcus* sp. The same symbiont was found capable of forming associations with a variety of organisms, thus opening up the possibility of consortial interconnections. Nevertheless, it must also be recognised that these dinoflagellates, radiolarian and tintinnid symbioses are very low in abundance and generally confined to the upper 50 m of the ocean.

Another approach to the study of biological interactions is to consider the *in situ* occurrence of groups of species that share similar requirements or even show proto-cooperative interaction. The natural co-occurrence and simultaneous increase or decrease in the numbers of some species may indicate the existence of 'functional associations' that help us to interpret and predict their dynamics (Reynolds et al. 2002). The supposition at the base of such associations is that common morphological or physiological properties offer relative, dynamic advantages of component species of the association. A new association was proposed that comprises *Synechococcus* spp. and potentially mixotrophic flagellates e.g. *Rhodomonas lacustris, Ceratium hirundinella, Cryptomonas erosa* (Callieri et al. 2006). Co-occurrence of Pcy and *Ceratium* spp. has been reported from mesotrophic lakes (Kasprzak et al. 2000), from Lake Kinneret (Berman et al. 1992) and Lake Maggiore (Callieri et al. 2006) (Fig. 8.28). In the latter lake, a 3-year study showed a phase of co-existence in which the organisms might each benefit from the association, followed by a phase of predation in which one member of the association prevailed over the other (Fig. 8.29). At low levels of physical and biological disturbance, the cycle can restart with prey recovery driven by nutrient excretion of phagotrophs. The association indicates that assemblages that form a functional group may not only have similar adaptations and requirements, but can exhibit predator–prey interactions, as was shown in a marine lagoon in France where the quasi simultaneous appearance of both Pcy and the dinoflagellate *Alexandrium catenella* was

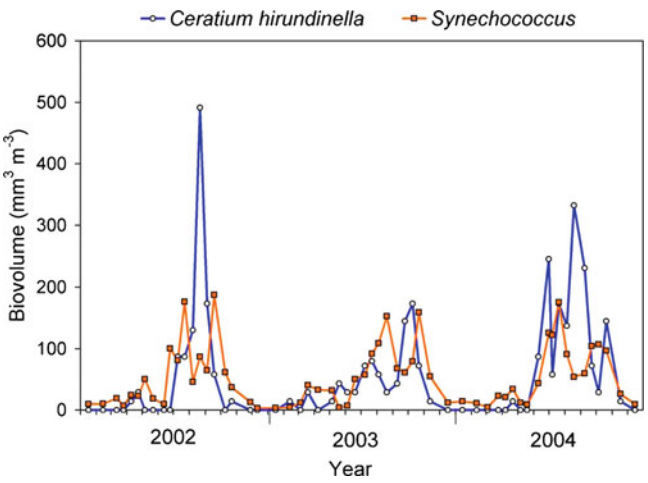

Fig. 8.29 Seasonal dynamics (years: 2002, 2003, 2004) of *Synechococcus* spp. and *Ceratium hirundinella* biovolume, in Lake Maggiore (From Callieri et al. 2006, modified)

observed (Collos et al. 2009). These authors hypothesised that Pcy can make up for a particulate nitrogen form during periods of limiting nutrients, thus providing *A. catenella* an ecological advantage over strictly autotrophic phytoplankton. The co-domination of a desirable prey organism, such as *Synechococcus* with its potential grazers opens up new perspectives on the interaction between the ecological categories of phytoplankton and the components of the microbial food web. We cannot refrain from conjecturing that these functional associations may be an advanced phase of a symbiotic association of cyanobacteria with eukaryotic plankton hosts, similar to those observed in the ocean (Carpenter and Foster 2002; Foster et al. 2006).

In our consideration of biological interactions, it is also opportune to refer to viral infections, as it is widely demonstrated that prokaryotic viruses can influence cellular organisms stronger than previously thought (Weinbauer 2004). The occurrence of viruses that infect *Synechococcus* is widespread and there is agreement that phages exert a significant selection pressure on *Synechococcus* (Mann 2003). Cyanophages are ubiquitous in aquatic environments, and can occur at abundances in excess of 10^6 mL^{-1} (Suttle 2000). Findings indicate that cyanophage infections can exert a major influence on the direction of Pcy succession in the sea (Mühling et al. 2005), and that marine viruses can act as intermediates for exchanging genes (Zeidner et al. 2005). Transduction is the phage-mediated gene transfer between a donor and a host, and has been recognized as is an important factor for bacterial evolution (e.g. Doolittle 1999). In three peri-alpine lakes viral impact on Pcy exceeded predation in autumn, but was highly variable throughout the early season (Personnic et al. 2009b). However, the interplay between viruses and nanoflagellates and their control of prokaryotes is not completely understood, largely due to a lack of knowledge of the direct interactions of viruses on predators and vice-versa (Jacquet et al. 2007; Pradeep Ram and Sime-Ngando 2008; Massana et al. 2007).

The extent of lysogeny in 19 freshwater *Synechococcus* strains indicated a high level within PC-rich *Synechococcus* (Dillon and Parry 2008). These authors found that the majority of cyanophages in the eutrophic lake they studied were temperate, that is they exist in a lysogenic association with their hosts. In the majority of the strains cell lysis by the phage was only triggered by an inducing agent used experimentally to assess the level of temperate phage infection in the host population. On the other hand viral DNA might also have a protective role on the host (Bailey et al. 2004). An example is provided by the discovery of a cyanophage encoding polypeptide D1 and D2 of the PSII, inducing repair cycles after photo-damage (Bailey et al. 2004). Nevertheless it is not known at which extent the phage modifies the properties of PSII and therefore manipulates the photosynthetic physiology of the infected cells.

Finally it is interesting to note the changing perceptions of cyanophages and other viruses in recent years; that is they are no longer seen as universally pernicious parasites, but as catalysts of information transfer and sustainers of the microbial web of energy transfer and matter cycling in aquatic ecosystems (Weinbauer 2004; Chap. 21).

8.5 Conclusions

Important advances in our perception of the significance of picocyanobacteria in freshwaters and oceans have occurred only within the last few decades, and these findings have come largely from an improved understanding of phylogenetic evolution of this major group. We now know that evolution of earliest cyanobacterial lineages were not marine, but likely were of terrestrial or freshwater origin and were unicellular. New paradigms in microbial ecology are now founded on a worldwide appreciation of functionally evolving clades and on genetically definable ecotypes. Taxonomic studies of phenotypic diversity are now coupled with genotypic diagnoses that can confirm whether similar phenotypes are phyletically close or due to convergent evolution.

The genera *Synechococcus* and *Cyanobium* are the dominant picocyanobacteria of fresh waters, while *Prochlorococcus* is typically marine. The phylogenetic analysis of the 16S rDNA sequences indicates that *Synechococcus* is polyphyletic. Despite some remaining uncertainties about the phylogenetic evolution of *Synechococcus*, we know that there are at least seven clusters of non-marine picocyanobacteria that have been found within the picophytoplankton clade, and that PE-rich spectral phenotype does not appear to be a general character that can be used to define a clade. Indeed, it has been found that phylogenies based on

phycobiliprotein genes are not easily comparable with 16S rRNA phylogeny and reveal a high diversity of *Synechococcus* strains. The adaptation of different ecological niches can be related to the highly variable number of horizontally acquired genes located in highly variable genomic regions or islands. This discovery opens new perspectives to the understanding of the local adaptation and the definition of species within the *Synechococcus* group. Further, it appears that the form-genus *Synechococcus* likely represents the ancestral morphology from which other types, including colonial forms, evolved.

To what extent the formation of microcolonies is due to the presence of specific *Synechococcus* genotypes or is the result of survival strategy is under debate. Here we consider microcolonies as transition forms from single-cell to colonial morphotypes. Grazing and UVR have been indicated as important factors which could regulate the equilibrium between single cells versus microcolonies or even larger colonial morphologies. The current challenge is to better understand the relationship between the diversity and ecology of Pcy, microcolonies and colonial Pcy and their interaction with the environmental factors that allow the proliferation of the most competitive genotypes.

Compared to our understanding of the physiology and ecology of Pcy, similar studies of colonial pico-cyanobacteria in lakes are sparse, and most published reports are focused on taxonomy rather than ecology. The picture emerging is that many colonial Pcy are part of the metaphyton community, loosely associated with littoral and benthic sediment and macrophytes, but capable of movement by currents, waves to the pelagic habitat where they experience growth and reproduction as plankton.

The morphometry and trophic state of lakes and ponds strongly influences composition, diversity and abundance of Pcy communities, most notably the relative distribution of Pcy PE-rich versus PC-rich cells. Light is known to be an important factor in niche differentiation of Pcy, and so also is in upper, mixed-layer depth, that can exert a strong influence on nutrient flux and lake trophic state. Lake nutrient status in turn directly affects Pcy presence and abundance relative to larger phytoplankton. The vast majority of lake studies now confirm that of total phytoplankton biomass the percentage contributed by Pcy increases with decreasing trophic state; in this sense there is a common ecological response of freshwater and marine Pcy, despite their phylogenetic differences. The acceptance of the validity of this empirical model can partially be explained by the high affinity of *Synechococcus* for orthophosphate, and its ability to utilise, at low PO_4^{3-} concentrations, organic sources of phosphate.

Protozoa grazing can influence the characteristics of Pcy communities and lead to changes in their structural and taxonomic composition. The knowledge of the mechanism of Pcy consumption and excretion/digestion is species-specific both for prey and predator. Ciliates and nanoflagellates can also serve as a trophic link between Pcy production and *Daphnia* production, thereby upgrading the nutritional value of Pcy as a food source by producing essential lipids such as sterols. The co-domination of a desirable prey organism, such as *Synechococcus* with its potential grazers opens up new perspectives on the interaction between the ecological categories of phytoplankton and the components of the microbial food web.

Adaptation to a changing environment, with phasing of fluctuating events, can subject the community to dominance by the fittest and most adaptive available species. These concepts should be evaluated in light of the new conceptual framework of community ecology, the meta-community, which considers the communities as shaped at different spatial scales (local and regional). Therefore in the debate on biotic versus abiotic regulation of community structure and dynamics we need to consider that local communities are not isolated but are linked by dispersal of multiple, potentially interactive, species.

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References

Adams DG (2000) Symbiotic interactions. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 523–561, 668 pp

Ahlgren NA, Rocap G (2006) Culture isolation and culture-independent clone libraries reveal new marine *Synechococcus* ecotypes with distinctive light and N physiologies. Appl Environ Microbiol 72:7193–7204

Ahlgren NA, Rocap G, Chisholm SW (2006) Measurement of *Prochlorococcus* ecotypes using real-time polymerase chain reaction reveals different abundances of genotypes with similar light physiologies. Environ Microbiol 8:441–454

Allende L, Izaguirre I (2003) The role of physical stability on the establishment of steady states in the phytoplankton community of two Maritime Antarctic lakes. Hydrobiologia 502:211–224

Ålvik G (1934) Plankton-Algen norwegischer Austernpollen I. Systematik und Vorkommen der Arten. Bergens Mus Årb 1934(6):47 pp

Arndt H (1993) Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) – a review. Hydrobiologia 255(256):231–246

Bailey S, Clokie MRJ, Millard A, Mann NH (2004) Cyanophage infection and photoinhibition in marine cyanobacteria. Mini-review. Res Microbiol 155:720–725

Balseiro EG, Modenutti BE, Queimaliños CP (1997) Nutrient recycling and shifts in N:P ratio by different zooplankton structures in a South Andes lake. J Plankton Res 19:805–817

Balseiro EG, Queimaliños C, Modenutti BE (2004) Grazing impact on autotrophic picoplankton in two south Andean lakes (Patagonia, Argentina) with different light:nutrient ratios. Rev Chil Hist Nat 77:73–85

Beardall J, Raven JA (2004) Potential effects of global change on microalgal photosynthesis, growth and ecology. Phycologia 43:26–40

Bec A, Martin-Creuzburg D, von Elert E (2006) Trophic upgrading of autotrophic picoplankton by the heterotrophic nanoflagellate *Paraphysomonas* sp. Limnol Oceanogr 51:1699–1707

Becker S, Böger P, Oehlmann R, Ernst A (2000) PCR bias in ecological analysis: a case study for quantitative Taq nuclease assays in analyses of microbial communities. Appl Environ Microbiol 66:4945–4953

Becker S, Fahrbach M, Böger P, Ernst A (2002) Quantitative tracing, by Taq nuclease assays, of a *Synechococcus* ecotype in a highly diversified natural population. Appl Environ Microbiol 68:4486–4494

Becker S, Singh AK, Postius C, Böger P, Ernst A (2004) Genetic diversity and distribution of periphytic *Synechococcus* spp. in biofilms and picoplankton of Lake Constance. FEMS Microbiol Ecol 49:181–190

Becker S, Richl P, Ernst A (2007) Seasonal and habitat-related distribution pattern of *Synechococcus* genotypes in Lake Constance. FEMS Microbiol Ecol 62:64–67

Bell JL (1991) Patches and picoplankton. Effects on larval life spans on gastropod larvae. Am Zool 31:6A

Bell T, Kalff L (2001) The contribution of picophytoplankton in marine and freshwater systems of different trophic status and depth. Limnol Oceanogr 46:1243–1248

Bell RT, Tranvik L (1993) Impact of acidification and liming on the microbial ecology of lakes. Ambio 22:325–330

Belykh OI, Ekaterina G, Sorokovikova T, Saphonova A, Tikhonova V (2006) Autotrophic picoplankton of Lake Baikal: composition, abundance and structure. Hydrobiologia 568:9–17

Bergman E, Hamrin SF, Romare P (1999) The effect of cyprinid reduction on the fish community. In Hansson LA and Bergman B (eds) Nutrient reduction and biomanipulation as tools to improve water quality. The Lake Ringsjön story. Hydrobiologia 404:65–75

Berman T, Yacobi YZ, Pollingher U (1992) Lake Kinneret phytoplankton: stability and variability during twenty years (1970–1989). Aquat Sci 54:104–127

Bertilsson S, Berglund O, Karl DM, Chisholm SW (2003) Elemental composition of marine *Prochlorococcus* and *Synechococcus*: implications for the ecological stoichiometry of the sea. Limnol Oceanogr 48:1721–1731

Bertoni R, Piscia R, Callieri C (2004) Horizontal heterogeneity of seston, organic carbon and picoplankton in the photic zone of Lago Maggiore, Northern Italy. J Limnol 63:244–249

Bird DJ, Kalff J (1987) Algal phagotrophy: regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae). Limnol Oceanogr 32:277–284

Bird C, Wyman M (2003) Nitrate/nitrite assimilation system of the marine picoplanktonic cyanobacterium *Synechococcus* sp. strain WH8103: effect of nitrogen source and availability on gene expression. Appl Environ Microbiol 69:7009–7018

Björk S et al (1972) Ecosystem studies in connection with the restoration of lakes. Verh Int Ver Limnol 18:379–387

Bláha L, Marsálek B (1999) Microcystin production and toxicity of picocyanobacteria as a risk factor for drinking water treatment plants. Algol Stud 92:95–108

Blank CE, Sánchez-Baracaldo P (2010) Timing of morphological and ecological innovations in the cyanobacteria – a key to understanding the rise in atmospheric oxygen. Geobiology 8:1–23

Blomqvist P (1996) Late summer phytoplankton responses to experimental manipulations of nutrients and grazing in unlimed and limed Lake Njupfatet, central Sweden. Arch Hydrobiol 137:425–455

Boenigk J, Matz C, Jürgens K, Arndt H (2001) The influence of preculture conditions and food quality on the ingestion and digestion process of three species of heterotrophic nanoflagellates. Microb Ecol 42:168–176

Boersma M, Wiltshire K (2006) Gut passage of phosphorus-limited algae through *Daphnia*: do they take up nutrients in the process? Arch Hydrobiol 167:498–500

Bourrelly P (1985) Les Algues d'Eaux Douce. III. Les Algues Bleues et Rouges, Les Eugléniens, Peridiniens et Cryptomonadines. Société Nouvelle des Éditions Boubée, Paris, 606 pp

Buma AGJ, van Hannen EJ, Veldhuis MJW, Gieskes WWC (1995) Monitoring ultraviolet B-induced DNA damage in individual diatom cells by immuno-fluorescent-thymin dimer detection. J Phycol 31:314–321

Burns CW, Schallenberg M (1996) Relative impacts of copepods, cladocerans and nutrients on the microbial food web of a mesotrophic lake. J Plankton Res 18:683–714

Burns CW, Schallenberg M (2001) Short-term impacts of nutrients, *Daphnia*, and copepods on microbial food-webs on an oligotrophic and eutrophic lake. N Z J Mar Freshw Res 35:695–710

Butcher RW (1952) Contributions to our knowledge of smaller marine algae. J Mar Biol Assoc UK 31:610–652

Callieri C (1996) Extinction coefficient of red, green and blue light and its influence on Pcy types in lakes at different trophic levels. Mem Ist Ital Idrobiol 54:135–142

Callieri C (2008) Picophytoplankton in freshwater ecosystems: the importance of small-sized phototrophs. Freshw Rev 1:1–28

Callieri C (2010) Single cells and microcolonies of freshwater picocyanobacteria: a common ecology. J Limnol 69:257–277

Callieri C, Bertoni R (1999) Organic carbon and microbial food web assemblages in an oligotrophic alpine lake. In: Straškrabová V, Callieri C, Fott J (eds) Pelagic food web in Mountain Lakes. MOuntain LAkes Research Program. J Limnol 58:136–143

Callieri C, Pinolini ML (1995) Picoplankton in Lake Maggiore, Italy. Int Rev Ges Hydrobiol 80:491–501

Callieri C, Piscia R (2002) Photosynthetic efficiency and seasonality of autotrophic picoplankton in Lago Maggiore after its recovery. Freshw Biol 47:941–956

Callieri C, Stockner JG (2002) Freshwater autotrophic picoplankton: a review. J Limnol 61:1–14

Callieri C, Amicucci E, Bertoni R, Vörös L (1996a) Fluorometric characterization of two picocyanobacteria strains from different underwater light quality. Int Rev Ges Hydrobiol 81:13–23

Callieri C, Bertoni R, Amicucci E, Pinolini ML, Jasser I (1996b) Growth rates of freshwater picocyanobacteria measured by FDC: problems and potentials for the estimation of picoplankton organic carbon synthesis. Arch Hydrobiol Spec Issues Adv Limnol 48:93–103

Callieri C, Lami A, Bertoni (2011) Microcolony formation by single-cell *Synechococcus* strains as a fast response to UV radiation. Appl Environ Microbiol 77:7533–7540

Callieri C, Morabito G, Huot Y, Neal P, Lichman E (2001) Photosynthetic response of pico- and nanoplanktonic algae to UVB, UVA and PAR in a high mountain lake. Aquat Sci 63:286–293

Callieri C, Karjalainen SM, Passoni S (2002) Grazing by ciliates and heterotrophic nanoflagellates on picocyanobacteria in Lago Maggiore, Italy. J Plankton Res 24:785–796

Callieri C, Balseiro E, Bertoni R, Modenutti B (2004) Picocyanobacterial photosynthetic efficiency under *Daphnia* grazing pressure. J Plankton Res 26:1471–1477

Callieri C, Moro S, Caravati E, Crosbie ND, Weisse T (2005) Strain specific photosynthetic response of freshwater picocyanobacteria. Verh Int Ver Limnol 29:777–782

Callieri C, Caravati E, Morabito G, Oggioni A (2006) The unicellular freshwater cyanobacterium *Synechococcus* and mixotrophic flagellates: evidence for a functional association in an oligotrophic, subalpine lake. Freshw Biol 51:263–273

Callieri C, Modenutti B, Queimaliños C, Bertoni R, Balseiro E (2007) Production and biomass of picophytoplankton and larger autotrophs in Andean ultraoligotrophic lakes: differences in light harvesting efficiency in deep layers. Aquat Ecol 80:345–362

Callieri C, Caravati E, Corno G, Bertoni R (2012) Picocyanobacterial community structure and space-time dynamics in the subalpine Lake Maggiore (N. Italy). J Limnol 71:95–103

Camacho A, Picazo A, Miracle MR, Vicente E (2003) Spatial distribution and temporal dynamics of picocyanobacteria in a meromictic karstic lake. Algol Stud 109:171–184

Campbell L, Carpenter EJ (1986a) Diel pattern of cell division in marine *Synechococcus* spp. (Cyanobacteria): use of frequency of dividing cell technique to measure growth rate. Mar Ecol Prog Ser 32:139–148

Campbell L, Carpenter EJ (1986b) Estimating the grazing pressure of heterotrophic nanoplankton on *Synechococcus* spp. using the seawater dilution and selective inhibitor techniques. Mar Ecol Prog Ser 33:121–129

Caravati E, Callieri C, Modenutti B, Corno G, Balseiro E, Bertoni R, Michaud L (2010) Picocyanobacterial assemblages in ultraoligotrophic Andean lakes reveal high regional microdiversity. J Plankton Res 32:357–366

Caron DA, Lim EL, Miceli G, Waterbury JB, Valois FW (1991) Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and coastal plankton community. Mar Ecol Prog Ser 76:205–217

Carpenter EJ, Foster RA (2002) Marine symbioses. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 11–18

Carreto JI, Carignan MO, Daleo G, De Marco SG (1990) Occurrence of mycosporine-like amino acids in the red tide dinoflagellate *Alexandrium escavatum*: UV-photoprotective compounds? J Plankton Res 12:909–921

Carrillo P, Reche I, Cruz-Pizarro L (1996) Quantification of the phosphorus released by zooplankton in an oligotrophic lake (La Caldera, Spain) – regulating factors and adjustment to theoretical-models. J Plankton Res 18:1567–1586

Chen F, Wang K, Kan JJ, Suzuki MT, Wommack KE (2006) Diverse and unique Pcy in Chesapeake Bay, revealed by 16S-23S rRNA internal transcribed spacer sequences. Appl Environ Microbiol 72:2239–2243

Chisholm SW (1992) Phytoplankton size. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum Press, New York, pp 213–237

Chisholm SW, Armbrust EV, Olson RJ (1986) The individual cell in phytoplankton ecology: cell cycle and applications of flow cytometry. Can Bull Fish Aquat Sci 214:343–369

Christoffersen K (1994) Variation of feeding activities of heterotrophic nanoflagellates on picoplankton. Mar Microb Food Web 8:111–123

Coleman ML, Sullivan MB, Martiny AC, Steglich C, Barry K, Delong EF, Chisholm SW (2006) Genomic islands and the ecology and evolution of *Prochlorococcus*. Science 311:1768–1770

Collos Y, Bec B, Jauzein C, Abadie E, Laugier T, Lautier J, Pastoureaud A, Souchu P, Vaquer A (2009) Oligotrophication and emergence of picocyanobacteria and a toxic dinoflagellate in Thau Lagoon, southern France. J Sea Res 61:68–75

Cronberg G (1988) *Cyanodictyon tubiforme*, a new chroococcal bluegreen alga from Lake Börringesjön, Scania, Sweden. Algol Stud 50–53:191–194

Cronberg G (1991) *Cyanothamnos plancticus* gen. Et sp. Nov., a new colonial cyanophyte from an eutrophic Scanian lake, Sweden. Algol Stud 64:61–70

Cronberg G (1999) Qualitative and quantitative investigations of phytoplankton in Lake Ringsjön, Scania Sweden. Hydrobiologia 404:27–40

Cronberg G (2003) New and interesting cyanoprokaryotes from temperate, brackish ponds and the Baltic Sea. Algol Stud 109:197–211

Cronberg G, Komárek J (1994) Planktic Cyanoprokaryotes found in South Swedish lakes during the XIIth international symposium on Cyanophyte research, 1992. Algol Stud 75:323–352

Cronberg G, Weibull C (1981) *Cyanodictyon imperfectum* a new chroococcal blue-green alga from Lake Trummen, Sweden. Algol Stud 27:101–110

Crosbie ND, Pöckl M, Weisse T (2003a) Dispersal and phylogenetic diversity of nonmarine picocyanobacteria, inferred from 16S rRNA gene and *cpc*BA-intergenic spacer sequence analyses. Appl Environ Microbiol 69:5716–5721

Crosbie ND, Pöckl M, Weisse T (2003b) Rapid establishment of clonal isolates of freshwater autotrophic picoplankton by single-cell and single-colony sorting. J Microbiol Method 55:361–370

Crosbie ND, Teubner K, Weisse T (2003c) Flow-cytometric mapping provides novel insights into the seasonal and vertical distributions of freshwater autotrophic picoplankton. Aquat Microb Ecol 33:53–66

Diaz M, Pedrozo F, Reynolds C(S), Temporetti P (2007) Chemical composition and the nitrogen-regulated trophic state of Patagonian lakes. Limnologica 37:17–27

Dillon A, Parry JD (2008) Characterization of temperate cyanophages active against freshwater phycocyanin-rich *Synechococcus* spp. Freshw Biol 53:1253–1261

Dillon A, Parry JD (2009) Amoebic grazing of freshwater *Synechococcus* strains rich in phycocyanin. FEMS Microbiol Ecol 69:106–112

Dillon JG, Tatsumi CM, Tandingan PG, Castenholz RW (2002) Effect of environmental factors on the synthesis of scytonemin, a UV-screening pigment, in a cyanobacterium (*Chroococcidiopsis* sp.). Arch Microbiol 177:322–331

Domingos P, Rubim TK, Molica RJR, Azevedo SMFO, Carmichael WW (1999) First report of microcystin production by picoplanktic cyanobacteria isolated from a Northeast Brazilian drinking water supply. Environ Toxicol 14:13–35

Doolittle WF (1999) Phylogenetic classification and the universal tree. Science 284:2124–2129

Drakare S, Blomqvist P, Bergström AK, Jansson M (2003) Relationships between picophytoplankton and environmental variables in lakes along a gradient of water colour and nutrient content. Freshw Biol 48:729–740

Drews G, Prauser H, Uhlmann D (1961) Massenvorkommen von *Synechococcus plancticus* nov. spec., einer solitären, planktischen Cyanophyceae, in einem Abwasserteich. Betrag zur Kenntnis der sogenannten "μ-Algen". Arch Mikrobiol 39:101–115

Dufresne A, Ostrowski M, Scanlan DJ, Garczarek L, Mazard S, Palenik BP, Paulsen IT, Tandeau de Marsac N, Wincker P, Dossat C, Ferriera S, Johnson J, Post AP, Hess WR, Partensky F (2008) Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. Genome Biol 9:R90. doi:10.1186/gb-2008-9-5-r90

DuRand MD, Olson RJ, Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. Deep Sea Res Part II 48:1983–2003

Ernst A (1991) Cyanobacterial picoplankton from Lake Constance I. solation by fluorescence characteristics. J Plankton Res 13:1307–1312

Ernst A, Postius C, Böger P (1996) Glycosylated surface proteins reflect genetic diversity among *Synechococcus* spp. of Lake Constance. Arch Hydrobiol 48:1–6

Ernst A, Becker S, Hennes K, Postius C (1999) Is there a succession in the autotrophic picoplankton of temperate zone lakes? In: Bell CR, Brylinski M, Johnson-Green P (eds) Microbial biosystems: new frontiers. Proceedings of the 8th international symposium on microbial ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada, pp 623–629

Ernst A, Becker S, Wollenzien VIA, Postius C (2003) Ecosystem dependent adaptive radiations of picocyanobacteria inferred from 16S rRNA and ITS-1 sequence analysis. Microbiology 149:217–228

Everroad RC, Wood AM (2006) Comparative molecular evolution of newly discovered picocyanobacterial strains reveals a phylogenetically informative variable region of beta-phycoerythrin. J Phycol 42:1300–1311

Fahnenstiel GL, Carrick HJ (1992) Phototrophic picoplankton in lakes Huron and Michigan: abundance, distribution, composition and contribution to biomass and production. Can J Fish Aquat Sci 49:379–388

Fahnenstiel GL, Patton TR, Carrick HJ, McCormick MJ (1991) Diel division cycle and growth rates of *Synechococcus* in lakes Huron and Michigan. Int Rev Ges Hydrobiol 76:657–664

Foster RA, Collier JL, Carpenter EJ (2006) Reverse transcription PCR amplification of cyanobacterial symbiont 16S rRNA sequences from single non-photosynthetic eukaryotic marine planktonic host cells. J Phycol 42:243–250

Frias-Lopez J, Thompson A, Waldbauer J, Chisholm S (2009) Use of stable isotope-labelled cells to identify active grazers of picocyanobacteria in ocean surface waters. Environ Microbiol 11:512–525

Fu FX, Warner ME, Zhang Y, Feng Y, Hutchins DA (2007) Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). J Phycol 43:485–496

Fuller NJ, Tarran GA, Yallop M, Orcutt KM, Scanlan DJ (2006) Molecular analysis of picocyanobacterial community structure along an Arabian sea transect reveals distinct spatial separation of lineages. Limnol Oceanogr 51:2515–2526

Furnas M, Crosbie ND (1999) In situ growth dynamics of the photosynthetic prokaryotic picoplankters *Synechococcus* and *Prochlorococcus*. Bull Inst Oceanogr Monaco N Spec 19:387–417

Furtado ALFF, Calijuri MDC, Lorenzi AS, Honda RY, Genuario DB, Fiore MF (2009) Morphological and molecular characterization of cyanobacteria from a Brazilian facultative wastewater stabilization pond and evaluation of microcystis production. Hydrobiologia 627:195–209

Gaedke U, Weisse T (1998) Seasonal and interannual variability of picocyanobacteria in Lake Costance. Arch Hydrobiol Spec Issues Adv Limnol 53:143–158

Gallager SM, Waterbury JB, Stoecker DK (1994) Efficient grazing and utilization of the marine cyanobacterium *Synechococcus* by larvae of the bivalve *Mercenaria mercenaria*. Mar Biol 119:251–259

Garcia-Pichel F (1994) A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. Limnol Oceanogr 39:1704–1717

Garcia-Pichel F, Castenholz RW (1991) Characterization and biological implications of scytonemin, a cyanobacteria sheath pigment. J Phycol 27:395–409

Garcia-Pichel F, Castenholz RW (1993) Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl Environ Microbiol 59:163–169

Geitler L (1932) Cyanophyceae. In: Rabenhorst L (ed) Kryptogamenflora, vol 14. Akademische Verlagsgesellschaft, Leipzig, 1069 pp

Gervais F, Padisák J, Koschel R (1997) Do light quality and low nutrient concentration favour picocyanobacteria below the thermocline of the oligotrophic Lake Stechlin? J Plankton Res 19:771

Gismervik I (2006) Top-down impact by copepods on ciliate numbers and persistence depends on copepod and ciliate species composition. J Plankton Res 28:499–507

Glibert PM, Ray RT (1990) Different patterns of growth and nitrogen uptake in two clones of marine *Synechococcus* spp. Mar Biol 107:273–280

Glover HE, Phinney DA, Yentsch CS (1985) Photosynthetic characteristics of picoplankton compared with those of larger phytoplankton populations in various water masses in the Gulf of Maine. Biol Oceanogr 3:223–248

Gophen M, Geller W (1984) Filter mesh size and food particle uptake by *Daphnia*. Oecologia 64:408–412

Gouvea AP, Boyer GL, Twiss MR (2008) Influeance of ultraviolet radiation, copper, and zinc on microcystin content in *Microcystis aeruginosa* (Cyanobacteria). Harmful Algae 7:194–205

Grossman AR, Schaefer MR, Chiang GG, Collier JL (1993) The phycobilisome, a light-harvesting complex responsive to environmental conditions. Microbiol Rev 57:725–749

Häder DP, Kumar HD, Smith RC, Worrest RC (2007) Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol Sci 6:267–285

Harris GP (1980) Temporal and spatial scales in phytoplankton ecology. Mechanisms, methods, models and management. Can J Fish Aquat Sci 37:877–900

Harrison JW, Smith REH (2009) Effects of ultraviolet radiation on the productivity and composition of freshwater phytoplankton communities. Photochem Photobiol Sci 8:1218–1232

Hauschild CA, McMurter HJG, Pick FR (1991) Effect of spectral quality on growth and pigmentation of picocyanobacteria. J Phycol 27:698–702

Havens KE, Heath RT (1991) Increased transparency due to changes in the algal size spectrum during experimental acidification in mesocosms. J Plankton Res 13:673–679

Haverkamp T, Acinas SG, Doeleman M, Stomp M, Huisman J, Stal LJ (2008) Diversity and phylogeny of Baltic Sea picocyanobacteria inferred from their ITS and phycobiliprotein operons. Environ Microbiol 10:174–188

Haverkamp THA, Schouten D, Doeleman M, Wollenzien U, Huisman J, Stal LJ (2009) Colorful microdiversity of *Synechococcus* strains (picocyanobacteria) isolated from the Baltic Sea. ISME J 3: 397–408

Hawley GRW, Whitton BA (1991a) Survey of algal picoplankton from lakes in five continents. Verh Int Ver Limnol 24:1220–1222

Hawley GRW, Whitton BA (1991b) Seasonal changes in chlorophyll-containing picoplankton populations of ten lakes in Northern England. Int Rev Ges Hydrobiol 76:545–554

Helbling EW, Villafañe VE, Barbieri ES (2001) Sensitivity of winter phytoplankton communities from Andean lakes to artificial ultraviolet-B radiation. Rev Chil Hist Nat 74:273–282

Helbling EW, Farías ME, Fernández Zenoff MV, Villafañe VE (2006) In situ responses of phytoplankton from the subtropical Lake La Angostura (Tucumán, Argentina) in relation to solar ultraviolet radiation exposure and mixing conditions. Hydrobiologia 559:123–134

Heldal M, Scanlan DJ, Norlans S, Thingstad F, Mann NH (2003) Elemental composition of single cells of various strains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis. Limnol Oceanogr 48:1731–1743

Hickel B (1985) *Cyanonephron styloides* gen. et sp. nov., a new chroococcal blue-green alga (Cyanophyta) from a brackish lake. Arch Hydrobiol Suppl 71; Algol Stud 38/39:99–104

Hickel B (1991) Two new chroococcal cyanophytes from a brackish environment, (Schlei-Fjord) Germany. Algol Stud 64:97–104

Hindák F (1975) Einige neue und interessante Planktonblaualgen aus der Westslowakei. Arch Hydrobiol Suppl 46(4); Algol Stud 13: 330–353

Hindák F (1982) On some planktonic coccoid blue-green algae characteristic by Fe-precipitates. Arch Hydrobiol Suppl 63(3); Algol Stud 32:241–258

Hindák F (1985) The cyanophycean genus *Lemmermanniella* Geitler 1942. Arch Hydrobiol Suppl 71(3); Algol Stud 40:393–401

Honda D, Yokota A, Sugiyama J (1999) Detection of seven major evolutionary lineages in cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences of five marine *Synechococcus* strains. J Mol Evol 48:723–739

Hopkinson BM, Morel FMM (2009) The role of siderophores in iron acquisition by photosynthetic marine microorganisms. Biometals 22:656–669

Houlahan JE, Currie DJ, Cottenie K, Cumming GS, Ernest SKM, Findlay CS, Fuhlendorf SD, Gaedke U, Legendre P, Magnuson JJ, McArdle BH, Muldavin EH, Noble D, Russell R, Stevens RD, Willis TJ, Woiwod IP, Wondzell SM (2007) Compensatory dynamics are rare in natural ecological communities. Proc Natl Acad Sci USA 104:3273–3277

Ilikchyan IN, McKay RML, Zehr JP, Dyhrman ST, Bullerjahn GS (2009) Detection and expression of the phosphonate transporter gene PHND in marine and freshwater picocyanobacteria. Environ Microbiol 11:1314–1324

Iturriaga R, Mitchell BG (1986) Chroococcoid cyanobacteria: a significant component in the food web dynamics of the open ocean. Mar Ecol Prog Ser 28:291–297

Ivanikova NV, Popels LC, McKay RML, Bullerjahn GS (2007) Lake Superior supports novel clusters of cyanobacterial picoplankton. Appl Environ Microbiol 73:4055–4065

Izaguirre I, Allende L, Marinone MC (2003) Comparative study of the planktonic communities of three lakes of contrasting trophic status at Hope Bay (Antarctic Peninsula). J Plankton Res 25:1079–1097

Jacquet S, Partensky F, Lennon JF, Vaulot D (2001) Diel patterns of growth and division in marine picoplankton in culture. J Phycol 37:357–369

Jacquet S, Domaizon I, Personnic S, Sime-Ngando T (2007) Do small grazers influence viral-induced bacterial mortality in Lake Bourget? Fund Appl Limnol 170:125–132

Jansson M, Olsson H, Pettersson K (1988) Phosphatases: origin, characteristic and function in lakes. Hydrobiologia 170:157–175

Jasser I (1997) The dynamics and importance of picoplankton in shallow, dystrophic lake in comparison with surface waters of two deep lakes with contrasting trophic status. Hydrobiologia 342–343:87–93

Jasser I, Arvola L (2003) Potential effects of abiotic factors on the abundance of autotrophic picoplankton in four boreal lakes. J Plankton Res 25:873–883

Jasser I, Królicka A, Karnkowska-Ishikawa A (2011) A novel phylogenetic clade of picocyanobacteria from the Mazurian lakes (Poland) reflects the early ontogeny of glacial lakes. FEMS Microbiol Ecol 75:89–98

Jezberová J, Komárková J (2007) Morphological transformation in a freshwater *Cyanobium* sp. induced by grazers. Environ Microbiol 9:1858–1862

Jochem FJ (2000) Probing the physiological state of phytoplankton at the single-cell level. Sci Mar 64:183–195

Joosten AMT (2006) Flora of the blue-green algae of the Netherlands. KNNV Publishing, Utrecht, p 239

Jürgens K, Jeppesen E (2000) The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake. J Plankton Res 22:1047–1070

Kana TM, Glibert PM (1987a) Effect of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechococcus* WH7803-I. Growth, pigmentation, and cell composition. Deep Sea Res 34:479–495

Kana TM, Glibert PM (1987b) Effect of irradiances up to 2000 μE m^{-2} s^{-1} on marine *Synechococcus* WH7803 -II. Photosynthetic responses and mechanisms. Deep Sea Res 34:497–516

Karentz D, McEuen FS, Land MC, Dunlap WC (1991) Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108: 157–166

Karl DM, Letelier R, Tupas L, Dore JE, Christian J, Hebel DV (1997) The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. Nature 388:533–538

Kasai F, Waiser MJ, Robarts RD, Arts MT (2001) Size dependent UVR sensitivity in Redberry lake phytoplankton communities. Ver Int Ver Limnol 27:2018–2023

Kasprzak P, Gervais F, Adrian R, Weiler W, Radke R, Jaeger I, Riest S, Siedel U, Schneider V, Boehme M, Eckmann R, Walz N (2000) Trophic characterization, pelagic food web structure and comparison of two mesotrophic lakes in Brandenburg (Germany). Int Rev Ges Hydrobiol 85:167–189

Katano T, Nakano S, Ueno H, Mitamura U, Anbutsu K, Kihira M, Satoh Y, Drucker V, Sugiyama M (2005) Abundance, growth and grazing loss rates of picophytoplankton in Barguzin Bay, Lake Baikal. Aquat Ecol 39:431–438

Katano T, Nakano S, Mitamura O, Yoshida H, Azumi H, Matsuura Y, Tanaka Y, Maezono H, Satoh Y, Satoh T, Sugiyama Y, Watanabe Y, Mimura T, Akagashi Y, Machida H, Drucker V, Tikhonova I, Belykh O, Fialkov VA, Han MS, Kang SH, Sugiyama M (2008) Abundance and pigment type composition of picocyanobacteria in Barguzin Bay, Lake Baikal. Limnology 9:105–114

Klut EM, Stockner JG (1991) Picoplankton associations in an ultra-oligotrophic lake on Vancouver Island, British Columbia. Can J Fish Aquat Sci 48:1092–1099

Koblížek M, Komenda J, Masojídek J, Pechar L (2000) Cell aggregation of the cyanobacterium *Synechococcus elongatus*: role of the electron transport chain. J Phycol 36:662–668

Komárek J (1958) Die taxonomische Revision der planktischen Blaualgen der Tschechoslowakei. In: Komárek J, Ettl H (eds) Algologische Studien. Naklad, ČSAV, Prague, pp 10–106

Komárek J (1976) Taxonomic review of the genera *Synechocystis* SAUV. 1892, *Synechococcus* Näg. 1849, and *Cyanothece* gen. nov. (Cyanophyceae). Arch Protistenk 118:119–179

Komárek J (1996) Towards a combined approach for the taxonomic and species delimitation of picoplanktic cyanoprokaryotes. Algol Stud 83:377–401

Komárek J, Anagnostidis K (1998) Cyanoprokaryota 1. Teil Chroococcales. Süsswasserflora von Mitteleuropas. Gustav Fischer, Stuttgart, 548 pp

Komárek J, Anagnostidis K (1999) Cyanoprokaryota 1. Teil Chroococcales. Süsswasserflora von Mitteleuropa 19/1. Gustav Fischer, Jena, 548 p

Komárek J, Kling H (1991) Variation in six planktonic cyanophyte genera in Lake Victoria (East Africa). Algol Stud 61:21–45

Komárek J, Komárková-Legnerová J (1992) Variability of some planktic gomphosphaeriod cyanoprocaryotes in northern lakes. Nord J Bot Sect Phycol 12:513–524

Komárek J, Azevedo SMFO, Domingos P, Komárková J, Tichý M (2001) Background of the Caruaru tragedy; a case taxonomic study of toxic cyanobacteria. Algol Stud 103:9–29

Komárek J, Cepák V, Kaštovský J, Sulek J (2004) What are the cyanobacterial genera *Cyanothece* and *Cyanobacterium*? Contribution to the combined molecular and phenotype taxonomic evaluation of cyanobacterial diversity. Algol Stud 113:1–36

Komárková J (2002) Cyanobacterial picoplankton and its colonial formations in two eutrophic canyon reservoirs (Czech Republic). Arch Hydrobiol 154:605–623

Komárková J, Cronberg G (1985) *Lemmermanniella pallida* (LEMM.) GEITL. from South-Swedish lakes. Arch Hydrobiol Suppl 71(3); Algol Stud 40:403–413

Komárková-Legnerová J, Cronberg G (1994) Planktic blue-green algae from lakes in South Scania Sweden Part I: Chroococcales. Algol Stud 72:13–51

Kranzler C, Lis H, Shaked Y, Keren N (2011) The role of reduction in iron uptake processes in a unicellular, planktonic cyanobacterium. Environ Microbiol 13:2990–2999

Lagerheim G (1883) Bidrag till Sveriges algflora. Öfv Kgl Vetensk-Akad Förhandl 40(2):37–39

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine microzooplankton. Mar Biol 67:283–288

Landry MR, Kirshtein J, Constantinou J (1995) A refined dilution technique for measuring the community grazing impact of microzooplankton with experimental tests in the Central Equatorial Pacific. Mar Ecol Prog Ser 120:53–63

Laurion I, Vincent WF (1998) Cell size vs. taxonomic composition as determinants of UV sensitivity in natural phytoplankton communities. Limnol Oceanogr 43:1774–1779

Lavallée BF, Pick FR (2002) Picocyanobacteria abundance in relation to growth and loss rates in oligotrophic to mesotrophic lakes. Aquat Microb Ecol 27:37–46

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzales A (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecol Lett 7:601–613

Lemmermann E (1904) Das Plankton schwedischer Gewasser. Ark Bot 2:1–209

Li WKW (1998) Annual average abundance of heterotrophic bacteria and *Synechococcus* in surface ocean waters. Limnol Oceanogr 43: 1743–1753

Liu H, Campbell L, Landry MR (1995) Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. Mar Ecol Prog Ser 116:277–287

Liu Z, Häder DP, Sommaruga R (2004) Occurrence of mycosporine-like aminoacids (MAAs) in the bloom-forming cyanobacterium *Microcyctis aeruginosa*. J Plankton Res 26:963–966

Liu X, Shi M, Liao Y, Gao Y, Zhang Z, Wen D, Wu W, An C (2006) Feeding characteristics of an amoeba (*Lobosea: Naegleria*) grazing upon cyanobacteria: food selection, ingestion and digestion process. Microb Ecol 51:315–325

Logue JB, Lindström ES (2008) Biogeography of bacterioplankton in inland waters. Freshw Rev 1:99–114

Mackey KRM, Paytan A, Grossman AR, Bailey S (2008) A photosynthetic strategy for coping in a high-light, low-nutrient environment. Limnol Oceanogr 53:900–913

Mackey KRM, Rivlin T, Grossman AR, Post AF, Paytan A (2009) Picophytoplankton responses to changing nutrient and light regimes during a bloom. Mar Biol 156:1535–1546

Maeda H, Kawai A, Tilzer MM (1992) The water bloom of cyanobacterial picoplankton in Lake Biwa, Japan. Hydrobiologia 248:93–103

Malinsky-Rushansky N, Berman T, Dubinsky Z (1995) Seasonal dynamics of picophytoplankton in Lake Kinneret, Israel. Freshw Biol 34:241–254

Mann KH (1993) Physical oceanography, food chains, and fish stocks: a review. ICES J Mar Sci 50:105–119

Mann NH (2003) Phages of marine cyanobacterial picophytoplankton. FEMS Microbiol Rev 27:17–34

Martin-Creuzburg D, Von Elert E (2006) Trophic upgrading of autotrophic picoplankton by the heterotrophic nanoflagellate *Paraphysomonas* sp. Limnol Oceanogr 51:1699–1707

Martin-Creuzburg D, Bec A, Von Elert E (2005) Trophic upgrading of picocyanobacterial carbon by ciliates for nutrition of *Daphnia magna*. Aquat Microb Ecol 41:271–280

Massana R, del Campo J, Dinter C, Sommaruga R (2007) Crash of a population of the marine heterotrophic flagellate *Cafeteria roenbergensis* by viral infection. Environ Microbiol 9:2660–2669

Mastala Z, Herodek S, V-Balogh K, Borbély G, Shafik HM, Vörös L (1996) Nutrient requirement and growth of a *Synechococcus* species isolated from Lake Balaton. Int Rev Ges Hydrobiol 81: 503–512

Meyer B (1994) A new species of *Cyanodictyon* (Cyanophyceae, Chroococcales) planktic in eutrophic lakes. Algol Stud 75:183–188

Mills MM, Ridame C, Davey M, La Roche J (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429:292–294

Modenutti BE, Balseiro EG (2002) Mixotrophic ciliates in an Andean lake: dependence on light and prey of an *Ophrydium naumanni* population. Freshw Biol 47:121–128

Modenutti BE, Queimaliños C, Balseiro EG, Reissig M (2003) Impact of different zooplankton structures on the microbial food web of a South Andean oligotrophic lake. Acta Oecol 24:S289–S298

Moore LR, Ostrowski M, Scanlan DJ, Feren K, Sweetsir T (2005) Ecotypic variation in phosphorus acquisition mechanisms within marine picocyanobacteria. Aquat Microb Ecol 39:257–269

Morel A, Bricaud A (1981) Theoretical results concerning light absorption in a discrete medium, an application to specific absorption of phytoplankton. Deep Sea Res 28:1375–1393

Morris I, Glover HE (1981) Physiology of photosynthesis by marine coccoid cyanobacteria some ecological implications. Limnol Oceanogr 26:957–961

Moser M, Callieri C, Weisse T (2009) Photosynthetic and growth response of freshwater picocyanobacteria are strain-specific and sensitive to photoacclimation. J Plankton Res 31:349–357

Moutin T, Thingstad TR, Van Wambeke F, Marie D, Slawyk G, Raimbault P, Claustre H (2002) Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus* ? Limnol Oceanogr 47:1562–1567

Mózes A, Présing M, Vörös L (2006) Seasonal dynamics of picocyanobacteria and picoeukaryotes in a large shallow lake (Lake Balaton, Hungary). Int Rev Ges Hydrobiol 91:38–50

Mühling M, Fuller NJ, Millard A, Somerfield PJ, Marie D, Wilson WH, Scanlan DJ, Post AF, Joint I, Mann NH (2005) Genetic diversity of marine *Synechococcus* and co-occurring cyanophage community: evidence for viral control of phytoplankton. Environ Microbiol 7:499–508

Murphy TP, Lean DRS, Nalewajko C (1976) Blue-green algae: their excretion of iron-selective chelators enables them to dominate other algae. Science 192:900–902

Nagata T, Takai K, Kawanobe K, Kim D, Nakazato R, Guselnikova N, Bondarenko N, Mologawaya O, Kostrnova T, Drucker V, Satoh Y, Watanabe Y (1994) Autotrophic picoplankton in southern Lake Baikal: abundance growth and grazing mortality during summer. J Plankton Res 16:945–959

Nagata T, Takay K, Kawabata K, Nakanishi M, Urabe J (1996) The trophic transfer via a picoplankton-flagellate-copepod food chain during a picocyanobacterial bloom in Lake Biwa. Arch Hydrobiol 137:145–160

Naumann E (1924) Sotvattnets plankton Stockholm, Sweden 267 pp

Nedoma J, Štrojsová A, Vrba J, Komárková J, Šimek K (2003) Extracellular phosphatase activity of natural plankton studied with ELF97 phosphate: fluorescence quantification and labelling kinetics. Environ Microbiol 5:462–472

Padisák J, Krienitz L, Koschel R, Nedoma J (1997) Deep-layer autotrophic picoplankton maximum in the oligotrophic Lake Stechlin, Germany: origin, activity, development and erosion. Eur J Phycol 32:403–416

Padisák J, Krienitz L, Scheffler W, Koschel R, Kristiansen J, Grigorszky I (1998) Phytoplankton succession in the oligotrophic Lake Stechlin (Germany) in 1994 and 1995. Hydrobiologia 369/370:179–197

Padisák J, Barbosa FAR, Koschel R, Krienitz L (2003) Deep layer cyanoprokaryota maxima are constitutional features of lakes: examples from temperate and tropical regions. Arch Hydrobiol Spec Issues Adv Limnol 58:175–199

Palenik B, Brahamsha B, Larimer FW, Land M, Hauser L, Chain P, Lamerdin J, Regala W, Allen EE, McCarren J, Paulsen I, Dufresne A, Partensky F, Webb EA, Waterbury J (2003) The genome of a motile marine *Synechococcus*. Nature 424:1037–1042

Passoni S, Callieri C (2000) Picocyanobacteria single forms, aggregates and microcolonies: survival strategy or species succession? Ver Int Ver Limnol 27:1879–1883

Pérez G, Quemaliños C, Modenutti B (2002) Light climate and plankton in the deep chlorophyll maxima in North Patagonian Andean lakes. J Plankton Res 24:591–599

Pernthaler J, Šimek K, Sattler B, Schwarzenbacher A, Bobkova J, Psenner R (1996) Short-term changes of protozoan control on autotrophic picoplankton in an oligo-mesotrophic lake. J Plankton Res 18:443–462

Personnic S, Domaizon I, Dorigo U, Berdjeb L, Jacquet S (2009a) Seasonal and spatial variability of virio-, bacterio-, and picophytoplanktonic abundances in three peri-alpine lakes. Hydrobiologia 627:99–116

Personnic S, Domaizon I, Sime-Ngando T, Jacquet S (2009b) Seasonal variations of microbial abundances and virus-versus flagellate-induced mortality of picoplankton in three peri-alpine lakes. J Plankton Res 31:1161–1177

Peštová D, Macek M, Martínez Pérez ME (2008) Ciliates and their picophytoplankton-feeding activity in a high-altitude warm-monomictic saline lake. Eur J Protistol 44:13–25

Pick FR (1991) The abundance and composition of freshwater picocyanobacteria in relation to light penetration. Limnol Oceanogr 36: 1457–1462

Pick FR, Agbeti DM (1991) The seasonal dynamic and composition of photosynthetic picoplankton communities in temperate lakes in Ontario, Canada. Int Rev Ges Hydrobiol 76:565–580

Ploug H, Stolte W, Jørgensen BB (1999) Diffusive boundary layers of the colony-forming plankton alga *Phaeocystis* sp. – implications for nutrient uptake and cellular growth. Limnol Oceanogr 44:1959–1967

Porter KG (1973) Selective grazing and differential digestion of algae by zooplankton. Nature 244:179–180

Porter KG (1975) Viable gut passage of gelatinous green algae ingested by *Daphnia*. Ver Int Ver Limnol 19:2840–2850

Postius C, Böger P (1998) Different interactions of phycoerythrin- and phycocyanin-rich *Synechococcus* spp. with diazotrophic bacteria from the picoplankton of Lake Constance. Arch Hydrobiol 141: 181–194

Postius C, Ernst A (1999) Mechanisms of dominance: coexistence of picocyanobacterial genotypes in a freshwater ecosystem. Arch Microbiol 172:69–75

Powell LM, Bowman JP, Skerratt JH, Franzmann PD, Burton HR (2005) Ecology of a novel *Synechococcus* clade occurring in dense populations in saline Antarctic lakes. Mar Ecol Prog Ser 291:65–80

Pradeep Ram AS, Sime-Ngando T (2008) Functional responses of prokaryotes and viruses to grazer effects and nutrient additions in freshwater microcosms. ISME J 2:498–509

Raven JA (1986) Physiological consequences of extremely small size for autotrophic organisms in the sea. In: Platt T, Li WKW (eds) Photosynthetic picoplankton. Department of Fisheries and Oceans, Ottawa. Can Bull Fish Aquat Sci 214:1–70

Reche I, Carrillo P, Cruz-Pizarro L (1997) Influence of metazooplankton on interactions of bacteria and phytoplankton in an oligotrophic lake. J Plankton Res 19:631–646

Reynolds CS, Huszar V, Kruk C, Naselli-Flores L, Melo S (2002) Towards a functional classification of the freshwater phytoplankton. J Plankton Res 24:417–428

Robertson BR, Tezuka N, Watanabe MM (2001) Phylogenetic analyses of *Synechococcus* strains (Cyanobacteria) using sequences of 16S rDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content. Int J Syst Evol Microbiol 51:861–871

Rocap G, Distel DL, Waterbury JB, Chisholm SW (2002) Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. Appl Environ Microbiol 68:1180–1191

Roff JC, Turner JT, Webber MK, Hopcroft RR (1995) Bacterivory by tropical copepod nauplii extent and possible significance. Aquat Microb Ecol 9:165–175

Ronneberger D (1998) Uptake of latex beads as size-model for food of planktonic rotifers. Hydrobiologia 387(388):445–449

Sánchez-Baracaldo P, Hayes PK, Blank CE (2005) Morphological and habitat evolution in the cyanobacteria using a compartmentalization approach. Geobiology 3:145–165

Sánchez-Baracaldo P, Handley BA, Hayes PK (2008) Picocyanobacterial community structure of freshwater lakes and the Baltic Sea revealed by phylogenetic analyses and clade-specific quantitative PCR. Microbiology 154:3347–3357

Sanders RW, Berninger UG, Lim EL, Kemp PF, Caron DA (2000) Heterotrophic and mixotrophic nanoplankton predation on picoplankton in the Sargasso Sea and Georges Bank. Mar Ecol Prog Ser 192:103–118

Sant'Anna CL, Azevedo MTP, Senna PAC, Komárková J, Komárková J (2004) Planktic cyanobacteria from Sao Paulo State, Brazil: Chroococcales. Rev Bras Bot 27(2):213–227

Scanlan DJ, West NJ (2002) Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. FEMS Microbiol Ecol 40:1–12

Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, Post AF, Hagemann M, Paulsen I, Partensky R (2009) Ecological genomics of marine picocyanobacteria. Microbiol Mol Biol Rev 73:249–299

Schindler DW (1977) Evolution of phosphorus limitation in lakes: natural mechanisms compensate for deficiencies of nitrogen and carbon in eutrophied lakes. Science 195:260–262

Schindler DW (1990) Experimental perturbations of the whole lakes as tests of hypotheses concerning ecosystem structure and function. Oikos 57:25–41

Schindler DW (2006) Recent advantages in understanding and management of eutrophication. Limnol Oceanogr 51:351–355

Shannon S, Chrzanowski T, Grover J (2007) Prey food quality affects flagellate ingestion rates. Microb Ecol 53:66–73

Sherr EB, Sherr BF (1993) Protistan grazing rates via uptake of fluorescently labeled prey. In: Kemp P, Sherr B, Sherr E, Cole J (eds) Handbook of methods in aquatic microbial ecology. Lewis Publisher, Boca Raton, pp 695–701

Sherr BF, Sherr EB, Albright LJ (1987) Bacteria: link or sink? Science 235:88–89

Sherr EB, Sherr BF, Berman T, Hadas O (1991) High abundance of picoplankton-ingesting ciliates during late fall in Lake Kinneret Israel. J Plankton Res 13:789–799

Šimek K, Chrzanowski TH (1992) Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. Appl Environ Microbiol 58:3715–3720

Šimek K, Bobkova J, Macek M, Nedoma J, Psenner R (1995) Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. Limnol Oceanogr 40:1077–1090

Šimek K, Macek M, Pernthaler J, Straskrabová V, Psenner R (1996) Can freshwater planktonic ciliates survive on a diet of picoplankton? J Plankton Res 18:597–613

Sime-Ngando T (1995) Population dynamics of autotrophic picoplankton in relation to environmental factors in a productive lake. Aquat Sci 57:91–105

Simon RD (1987) Inclusion bodies in the cyanobacteria: cyanophycin, polyphospate, polyhedral bodies. In: Fay P, Van Baalen C (eds) The cyanobacteria. Elsevier, Amsterdam/New York/Oxford, pp 199–225, 543 pp

Sinha RP, Häder DP (2008) UV-protectants in cyanobacteria. Plant Sci 174:278–289

Six C, Thomas JC, Garczarek L, Ostrowski M, Dufresne A, Blot N, Scanlan DJ, Partensky F (2007) Diversity and evolution of phycobilisomes in marine Synechococcus spp.: a comparative genomics study. Genome Biol 8:R259

Skuja H (1932) Vorarbeiten zu einer Algenflora von Lettland. Bibl Phycol 26:1–302

Skuja H (1948) Taxonomie des Phytoplanktons einiger Seen in Uppland, Schweden. Symb Bot Upsal 9:1–399

Skuja H (1964) Grundzüge der Algenflora und Algenvegetation der Fjeldgegenden um Abisko in schwedisch-Lappland. Nova Acta Reg Soc Sci Upsal Ser 4 18(3):1–465

Sommaruga R (2009) Perspectives and research on environmental effects of ultraviolet radiation. Photochem Photobiol Sci 8:1217

Sommaruga R, Hofer JS, Alonso-Sáez L, Gasol JM (2005) Differential sunlight sensitivity of picophytoplankton from surface Mediterranean coastal waters. Appl Environ Microbiol 71:2154–2157

Sommaruga R, Chen Y, Liu Z (2009) Multiple strategies of bloom-forming *Microcyctis* to minimize damage by solar ultraviolet radiation in surface waters. Microb Ecol 57:667–674

Søndergaard M (1991) Phototrophic picoplankton in temperate lakes: seasonal abundance and importance along a trophic gradient. Int Rev Ges Hydrobiol 76:505–522

Staley JT (1997) Biodiversity: are microbial species threatened? Curr Opin Biotechnol 8:340–345

Stanier RY, Kuniswawa R, Mandel R, Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (Order Chroococcales). Bacteriol Rev 35:171–205

Stockner JG (ed) (1991a) Autotrophic picoplankton in freshwater ecosystems. Int Rev Ges Hydrobiol 76:664 pp

Stockner JG (1991b) Autotrophic picoplankton in freshwater ecosystems: the view from the summit. Int Rev Ges Hydrobiol 76:483–492

Stockner JG (1998) Global warming, picocyanobacteria and fisheries decline: is there a connection? In: Atti del 12° Congresso AIOL, Vol.II, Genova, pp 29–37

Stockner JG, Antia NJ (1986) Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can J Fish Aquat Sci 43:2472–2503

Stockner JG, Porter KG (1988) Microbial food webs in fresh-water planktonic ecosystems. In: Carpenter SR (ed) Complex interactions in lake communities. Springer, New York, pp 69–83, 283 pp

Stockner JG, Shortreed KS (1988) Response of *Anabaena* and *Synechococcus* to manipulation of nitrogen:phosphorus ratios in a lake fertilization experiment. Limnol Oceanogr 33(1348):1361

Stockner JG, Shortreed KS (1989) Algal picoplankton production and contribution to food webs in oligotrophic British Columbia lakes. Hydrobiologia 173:151–166

Stockner JG, Shortreed KS (1991) Phototrophic picoplankton: community composition abundance and distribution across a gradient of oligotrophic British Columbia and Yukon Territory lakes. Int Rev Ges Hydrobiol 76:581–601

Stockner JG, Shortreed KS (1994) Autotrophic picoplankton community dynamics in a pre-alpine lake in British Columbia, Canada. Hydrobiologia 274:133–142

Stockner J, Callieri C, Cronberg G (2000) Picoplankton and other non-bloom forming cyanobacteria in lakes. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 195–238, 688 pp

Stockner JG, Langston A, Sebastian D, Wilson G (2005) The limnology of Williston Reservoir: British Columbia's largest lacustrine ecosystem. Water Qual Res J Can 40:28–50

Stomp M, Huisman J, de Jongh F, Veraart AJ, Gerla D, Rijkeboer M, Ibelings BW, Wollenzien UIA, Stal LJ (2004) Adaptive divergence in pigment composition promotes phytoplankton biodiversity. Nature 432:104–107

Stomp M, Huisman J, Vörös L, Pick FR, Laamanen M, Haverkamp T, Stal LJ (2007) Colorful coexistence of red and green picocyanobacteria in lakes and seas. Ecol Lett 10:290–298

Straškrabová V, Callieri C, Carrillo P, Cruz-Pizarro L, Fott J, Hartman P, Macek M, Medina-Sánchez JM, Nedoma J, Šimek K (1999a) Investigation on pelagic food web in mountain lakes – aims and methods. In: Straškrabová V, Callieri C, Fott J (eds) Pelagic food web in Mountain Lakes. MOuntain LAkes Research Program. J Limnol 58:77–87

Straškrabová V, Callieri C, Fott J (eds) (1999b) Pelagic food web in mountain lakes (Mountain Lakes Research Program). J Limnol 58:222 pp

Štrojsová A, Vrba J, Nedoma J, Komarková J, Znachor P (2003) Seasonal study of extracellular phosphatase expression in the phytoplankton of a eutrophic reservoir. Eur J Phycol 38:295–306

Sundt-Hansen LE, Olsen Y, Stibor H, Heldal M, Vadstein O (2006) Trophic cascades mediated by copepods, not nutrient supply rate, determine the development of picocyanobacteria. Aquat Microb Ecol 45:207–218

Suttle C (2000) Cyanophages and their role in the ecology of cyanobacteria. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 563–589, 668 pp

Szelag-Wasielewska E (2003) Phytoplankton community structure in non-stratified lakes of Pomerania (NW Poland). Hydrobiologia 506–509:229–236

Takano H, Arai T, Hirano M, Matsunaga T (1995) Effects of intensity and quality of light on phycocyanin production by a marine cyanobacterium *Synechococcus* sp. NKBG 042902. Appl Microbiol Biotechnol 43:1014–1018

Tarao M, Jezbera J, Hahn M (2009) Involvement of cell surface structures in size-independent grazing resistance of freshwater Actinobacteria. Appl Environ Microbiol 75:4720–4726

Tarbe AL, Unrein F, Stenuite S, Pirlot S, Sarmento H, Sinyinza D, Jean-Descy JP (2011) Protist herbivory: a key pathway in the pelagic food web of Lake Tanganyika. Microb Ecol 62:314–323

Taylor GT (1982) The role of pelagic heterotrophic protozoa in nutrient cycling: a review. Ann Inst Oceanogr 58:227–241

Timmermans KR, van der Wagt B, Veldhuis MJW, Maatman A, de Baar HJW (2005) Physiological responses of three species of marine pico-phytoplankton to ammonium, phosphate, iron and light limitation. J Sea Res 53:109–120

Toro M, Camacho A, Rochera C, Rico E, Bañón M, Fernandez-Valiente E, Marco E, Justel A, Avendañn MC, Ariosa Y, Vincent WF, Quesada A (2007) Limnological characteristics of freshwater ecosystems of Byers peninsula, Livingstone Island, in maritime Antarctica. Polar Biol 30:635–649

Tyrrell T (1999) The relative influences of nitrogen and phosphorus on oceanic primary production. Nature 400:525–531

Urbach E, Scanlan DJ, Distel DL, Waterbury JB, Chisholm SW (1998) Rapid diversification of marine picophytoplankton with dissimilar light-harvesting structure inferred from sequences of *Prochlorococcus* and *Synechococcus* (Cyanobacteria). J Mol Evol 46:188–201

Vadstein O (2000) Heterotrophic planktonic bacteria and cycling of phosphorus: phosphorus requirements, competitive ability, and food web interactions. In: Schink B (ed) Advances in microbial ecology, vol 16. Kluwer Academic Publisher, New York, pp 115–167

Van Donk E, Hessen DO (1993) Grazing resistance in nutrient-stressed phytoplankton. Oecologia 93:508–511

Van Donk E, Faafeng BA, De Lange HJ, Hessen DO (2001) Differential sensitivity to natural ultraviolet radiation among phytoplankton species in Arctic lakes (Spitsbergen, Norway). Plant Ecol 154:247–259

Van Gremberghe I, Van Wichelen J, Van der Gucht K, Vanormelingen P, D'hondt S, Boutte C, Wilmotte A, Vyverman W (2008) Covariation between zooplankton community composition and cyanobacterial community dynamics in Lake Blaarmeersen (Belgium). FEMS Microbiol Ecol 63:222–237

Van Mooy BAS, Rocap G, Fredericks HF, Evans CT, Devol AH (2006) Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. Proc Natl Acad Sci USA 103:8607–8612

Van Mooy BAS, Fredericks HF, Pedler BE, Dyhrman ST, Karl DM, Koblížek M, Lomas MW, Mincer TJ, Moore LR, Moutin T, Rappé MR, Webb EA (2009) Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarsity. Nature 458:69–72

Vázquez-Domínguez E, Peters F, Gasol JM, Vaqué D (1999) Measuring the grazing losses of picoplankton: methodological improvements in the use of fluorescently labeled tracers combined with flow cytometry. Aquat Microb Ecol 20:119–128

Vázquez-Domínguez E, Duarte CM, Agustí S, Jürgens K, Vaqué D, Gasol JM (2008) Microbial plankton abundance and heterotrophic activity across the Central Atlantic Ocean. Prog Oceanogr 79:83–94

Veldhuis MJW, Admiral W (1987) Influence of phosphate depletion on the growth and colony formation of *Phaeocystis pouchetii*. Mar Biol 95:47–54

Villafañe VE, Andrade M, Lairana V, Zaratti F, Helbling EW (1999) Inhibition of phytoplankton photosynthesis by solar ultraviolet radiation: studies in Lake Titicaca, Bolivia. Freshw Biol 42:215–224

Villafañe VE, Sundbäck K, Figueroa FL, Helbling EW (2003) Photosynthesis in the aquatic environment as affected by UVR. In: Helbling EW, Zagarese H (eds) UV effects in aquatic organisms and ecosystems. Comprehensive series in photochemistry and photobiology. The Royal Society of Chemistry, Cambridge, pp 357–398

Villafañe VE, Marcoval MA, Helbling EW (2004) Photosynthesis versus irradiance characteristics in phytoplankton assemblages off Patagonia (Argentina): temporal variability and solar UVR effects. Mar Ecol Prog Ser 284:23–34

Vincent WF (2000) Cyanobacteria dominance in the polar region. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 321–340, 668 pp

Vincent WF, Bowman JP, Rankin LM, McMeekin TA (2000) Phylogenetic diversity of picocyanobacteria in Arctic and Antarctic ecosystems. In: Bell CR, Brylinsky M, Johnson-Green P (eds) Microbial biosystems: new frontiers. Atlantic Canada Society for Microbial Ecology, Halifax, pp 317–322

Vörös L, Callieri C, Balogh KV, Bertoni R (1998) Freshwater picocyanobacteria along trophic gradient and light quality range. Hydrobiologia 369(370):117–125

Vörös L, Mózes A, Somogyi B (2009) A five-year study of autotrophic winter picoplankton in Lake Balaton, Hungary. Aquat Ecol 43: 727–734

Vrede K (1996) Regulation of bacterioplankton production and biomass in an oligotrophic clearwater lake – the importance of the phytoplankton community. J Plankton Res 18:1009–1032

Waterbury JB, Watson SW, Valois FW, Franks DG (1986) Biological and ecological characterisation of the marine unicellular cyanobacterium *Synechococcus*. Can Bull Fish Aquat Sci 214:17–120

Wehr JD (1993) Effects of experimental manipulations of light phosphorus supply on competition among picoplankton and nanoplankton in an oligotrophic lake. Can J Fish Aquat Sci 50:936–945

Weinbauer MG (2004) Ecology of prokaryotic viruses. FEMS Microbiol Rev 28:127–181

Weisse T (1990) Trophic interactions among heterotrophic microplankton, nanoplankton, and bacteria in Lake Constance (FRG). Hydrobiologia 191:111–122

Weisse T (1993) Dynamics of autotrophic picoplankton in marine and freshwater ecosystems. In: Jones JG (ed) Advances in microbial ecology, vol 13. Plenum Press, New York, pp 327–370

Weisse T, Kenter U (1991) Ecological characteristics of autotrophic picoplankton in a prealpine lake. Int Rev Ges Hydrobiol 76:493–504

Weisse T, Schweizer A (1991) Seasonal and interannual variation of autotrophic picoplankton in a large prealpine lake (Lake Constance). Verh Int Ver Limnol 24:821–825

West W, West GS (1894) On some algae from the West Indies. J Linn Soc 30(208):264–280

Whitton BA, Grainger SLJ, Hawley GRW, Simon JW (1991) Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb Ecol 21:85–98

Whitton BA, Al-Shehri AH, Ellwood NTW, Turner BL (2005) Ecological aspects of phosphatase activity in cyanobacteria, eukaryotic algae and bryophytes. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. Commonwealth Agricultural Bureau, Wallingford, pp 205–241, 399 pp

Wilhelm SW (1995) Ecology of iron-limited cyanobacteria: a review of physiological responses and implications for aquatic systems. Aquat Microb Ecol 9:295–303

Wilmotte A, Golubić S (1991) Morphological and genetic criteria in the taxonomy of Cyanophyta/Cyanobacteria. Algol Stud 64:1–24

Wilson DS (1992) Complex interactions in metacommunities, with implications for biodiversity and higher levels of selection. Ecology 73:1984–2000

Winder M (2009) Photosynthetic picoplankton dynamics in Lake Tahoe: temporal and spatial niche partitioning among prokaryotic and eukaryotic cells. J Plankton Res 31:1307–1320

Wood AM, Van Valen LM (1990) Paradox lost? On the release of energy-rich compounds by phytoplankton. Mar Microb Food Web 4:103–116

Wood AM, Horan PK, Muirhead K, Phinney DA, Yentsch CM, Waterbury JB (1985) Discrimination between types of pigments in marine *Synechococcus* spp. by scanning spectroscopy, epifluorescence microscopy and flow cytometry. Limnol Oceanogr 30:1303–1315

Yang Z, Kong F (2012) Formation of large colonies: a defense mechanism of *Microcystis aeruginosa* under continuous grazing pressure by flagellate *Ochromonas* sp. J Limnol 71:61–66

Yoshida T, Gurung TB, Kagami M, Urabe J (2001) Contrasting effects of cladoceran (*Daphnia galeata*) and calanoid copepod (*Eodiaptomus japonicus*) on algal and microbial plankton in a Japanese lake, Lake Biwa. Oecologia 129:602–610

Zaret M, Suffern KL (1976) Vertical migration in zooplankton as a predator avoidance mechanism. Limnol Oceanogr 21:804–813

Zeidner G, Bielawski JP, Shmoish M, Scanlan DJ, Sabehi G, Beja O (2005) Potential photosynthesis gene recombination between *Prochlorococcus* and *Synechococcus* via viral intermediates. Environ Microbiol 7:1505–1513

Zöllner E, Santer B, Boersma M, Hoppe HG, Jürgens K (2003) Cascading predation effects of *Daphnia* and copepods on microbial food web components. Freshw Biol 48:2174–2193

Zwirglmaier K, Heywood JL, Chamberlain K, Malcolm E, Woodward S, Zubkov MV, Scanlan DJ (2007) Basin-scale distribution patterns of picocyanobacterial lineages in the Atlantic Ocean. Environ Microbiol 9:1278–1290

Zwirglmaier K, Jardillier L, Ostrowski M, Mazard S, Garczarek L, Vaulot D, Not F, Massana R, Ulloa O, Scanlan DJ (2008) Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ Microbiol 10:147–161

Zwirglmaier K, Spence E, Zybkov MV, Scanlan DJ, Mann NH (2009) Differential grazing of two heterotrophic nanoflagellates on marine *Synechococcus* strains. Environ Microbiol 11:1767–1776

Cyanobacteria in Freshwater Benthic Environments

9

J. Thad Scott and Amy M. Marcarelli

Contents

J.T. Scott (✉)
Department of Crop, Soil and Environmental Sciences, Dale Bumpers College of Agricultural, Food and Life Sciences, University of Arkansas, Fayetteville, AR 72701, USA
e-mail: jts004@uark.edu

A.M. Marcarelli
Department of Biological Sciences, Michigan Technological University, 1400 Townsend Drive, Dow 740, Houghton, MI 49931, USA
e-mail: ammarcar@mtu.edu

Summary

Cyanobacteria are widespread in freshwater benthic environments, which include wetlands, lake littoral zones, streams and rivers. This chapter outlines the major constraints on cyanobacteria in these environments. Environmental and ecological factors that determine the diversity and biomass of cyanobacteria in the freshwater benthos include physical disturbance in the form of turbulent energy and wetting/drying cycles, temperature, light, nutrients and grazing. Nutrients are particularly important, because their concentrations can control cyanobacteria within and among benthic habitats, and cyanobacteria can reciprocally influence nutrient availability via nitrogen fixation and phosphorus co-precipitation by calcareous species. Top-down control via grazing may also help to explain diverse patterns of cyanobacterial abundance, because of the interactions which occur between the cyanobacteria and their predators. Anthropogenic activities sometimes have a pronounced effect on the environmental conditions that control cyanobacterial diversity and abundance in these habitats and the resulting functional changes in the communities can result in a loss of important ecosystem services provided by these organisms.

9.1 Introduction

Cyanobacteria can proliferate in shallow freshwater habitats where substantial light penetrates to benthic substrates. For example, *Nostoc* can occur on the bottom of shallow freshwater environments as loose colonies or firmly attached (Fig. 9.1). This chapter on benthic cyanobacteria is organized into three sections: (1) The major environmental and ecological controls on benthic cyanobacteria in freshwaters, (2) Examples of cyanobacteria in lentic and lotic habitats, largely based on research in North and Central America, and (3) Some of the major unresolved issues relating to these topics and what should be done to answer them.

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Fig. 9.1 *Nostoc* sp. growing on rocky bottom substrate of the Yankee Fork of the Salmon River, central Idaho (Photo courtesy of J. Ryan Bellmore)

Most cyanobacteria occur in consortia or communities that exhibit complex ecological interactions among species. The terms periphyton and biofilm are used to describe such communities of diverse microorganisms (Stevenson 1996a), although biofilm can also be applied to communities lacking any phototrophs. Periphyton may be attached to or closely associated with submerged plants, rocks and sediments, and the attached microbial assemblages for each of the substrates specifically are termed epiphyton, epilithon and epipelon, respectively. Loosely associated material of any of these can separate from the substrata and float to or near the surface of the water to generate floating microbial mats (Durako et al. 1982), termed metaphyton.

9.2 Environmental and Ecological Constraints

9.2.1 Physical Disturbance

What controls where and when cyanobacteria occur in freshwater benthic environments? In many instances the conditions that favour cyanobacteria here are similar to those that favour them in other environments, but there are some aspects of physical, chemical and biological conditions especially important for benthic communities (Table 9.1).

Conditions permitting, benthic community establishment is often interrupted by physical disturbances such as current velocity, wave action, and periodic drying and wetting. Stream currents can disturb the diffusive boundary layer of the benthic community, promoting rapid eddy nutrient diffusion (Riber and Wetzel 1987; Borchardt 1996), which can stimulate benthic cyanobacterial metabolism (Sperling and Hale 1973; Dodds 1989). However, at high velocities shear stress and turbulent energy increase, and rates of cell immigration decline (McIntire 1966), resulting in biomass sloughing and net biomass export.

An important predictor of the ability of cyanobacteria to resist shear stress is growth form (Peterson 1996): long filaments and loose mat forms may be susceptible to the stress imposed by current velocity (Grimm and Fisher 1989; Biggs et al. 1998), while adnate growth forms may be better able to withstand shear stress (Stevenson 1996b; Pringle and Hamazaki 1997). Cyanobacteria can also resist shear stress through basal structures that promote adhesion (Dudley and D'Antonio 1991; Pringle and Hamazaki 1997), dense mucilage production that buffers turbulence (Neumann et al. 1970), and low vertical profiles that reduce drag (Blenkinsopp and Lock 1994). In a 42-day experimental study in a mountain stream, Poff et al. (1990) found that diatoms seemed better able to withstand shear stress than *Oscillatoria* sp. and *Anabaena affinis*, filamentous cyanobacteria which only occurred at current velocities up to 20 cm s^{-1}. However, other cyanobacteria such as several *Chamaesiphon* spp. which have crustose growth forms are conspicuous in many streams with much higher current velocities (B.A.Whitton, 2009 personal communication). Variability in current velocity, turbulence, and age of community all influence the capacity of benthic phototrophs to withstand physical disturbance.

High flows, such as those occurring during flood events, may also negatively affect benthic cyanobacteria by physically moving the substrate (Douglas 1958), causing attached material to become dislodged. The amount of periphyton on a substrate often shows a positive relationship to the size of the substrate, because higher flows move larger rocks, as shown in a study of the River Necker, Switzerland (Uehlinger 1991) and mountain streams in the USA (Myers et al. 2007). However, fine suspended sediment can abrade epilithon from the surface of stable substrates, and studies have rarely differentiated between the effects of bed movement and abrasion in streams (but see Francoeur and Biggs 2006). In addition, the composition of the substrate could also have an effect, with potential differences between, for instance, a smooth granite and a rough calcareous sandstone surface.

Currents within lentic systems may have similar effects as those for streams, with resource delivery being important at low turbulent velocities (Stevenson and Stoermer 1981) and physical disruption by wave action causing abrasion and export at high turbulent velocities (Luttenton and Rada 1986; Peterson et al. 1990). However, the presence of macrophytes in shallow lentic systems may decrease substrate mobility through current dissipation and rooting. Carpenter and Lodge (1986) suggested that macrophytes aid in creating buffers to or refugia from physical disturbance in these systems, which attached cyanobacteria may utilize.

In addition to turbulence, periodic lack of water can greatly affect benthic cyanobacteria. Cyanobacterial species common to upland and even desert soil environments often occur in freshwater benthic communities subject to frequent desiccation

Table 9.1 Comparison of the relative importance and strength of environmental controls on cyanobacteria in various freshwater benthic habitats

Environmental factor	Wetlands	Lake Benthos	Streams
Physical disturbance	Moderate – wet/dry cycles	Low to moderate – wave activity	Very high – shear stress, bed disturbance, wet/dry cycles
Light	Can be limiting in mats and deep water, UV stress in shallow/drying water	Limiting in deep water, UV stress in shallow water	Limiting when shaded by canopy, otherwise high availability
Temperature	Ranges seasonally, regionally. Extreme diel variability at air-water interface	Ranges seasonally, regionally. Stable over short (e.g., diel) periods	Ranges seasonally, regionally, and locally. Can have large diel changes.
Nutrients	Loss of calcareous species and biomass with P enrichment	Competition between phytoplankton and periphyton can be severe – benthic diazotrophs may avoid competition	Effects may be secondary after light and grazing; low N conditions favour cyanobacteria
Grazing	Can be strong in mats, grazers can consume cyanobacteria effectively	Effects on benthos unclear, likely small because of high benthic food availability and low grazer densities	Strong top-down limitation, grazers may avoid cyanobacteria

(Gottlieb et al. 2006), and the relative proportion of their taxa increases as the desiccation period increases (Gottlieb et al. 2005). Cyanobacteria may dominate biofilms in streams with rapid wet-dry cycles (Robson et al. 2008). Cyanobacteria may be capable of rapidly resuming biofilm metabolism and may dominate algal communities after rewetting (Romani and Sabater 1997; Robson 2000). This ability to resist desiccation is often aided by an extracellular polysaccharide matrix such as a sheath or mucilage (Chap. 18).

9.2.2 Light

The light climate influences where cyanobacteria occur in freshwater benthic habitats on both macro- and micro-scales. Light climate variation at larger scales is influenced by shading from riparian vegetation or emergent or submerged macrophytes (Hill et al. 1995), colour and suspended matter in the water (Davies-Colley et al. 1992) and water body morphometry (Vadeboncoeur et al. 2008). Light availability controls diel patterns of cyanobacterial activity, including photosynthesis and N_2 fixation, as reported for two streams in California, USA (Horne 1975), an upland stream in Teesdale, UK (Livingstone et al. 1984), and a wetland in Texas, USA (Scott et al. 2007).

Light can be attenuated rapidly within a biofilm or microbial mat, with marked differences in the relative absorption of different spectral regions (Chaps. 4 and 19). This is reflected in vertical differences in the species composition within benthic communities, though horizontal differences may also be important. Vertical changes in species composition reflect differences in the physiological mechanisms for light capture and photosynthesis. For instance, cyanobacteria growing below a layer of diatoms in a microbial mat were able to absorb light not used by the diatoms (Jørgensen et al. 1987). The ability of cyanobacterial phycobilins to utilize long-wavelength light also helps to explain why cyanobacteria sometimes dominate under low-light conditions, such as near the bottom of the photic zone in lakes (Loeb and Reuter 1981; Reuter and Axler 1992) and in shaded streams (Bourassa and Cattaneo 2000). Conversely, diazotrophic cyanobacteria generally have higher irradiance requirements and maximum rates of photosynthesis and N_2 fixation can occur at the same depth in the pelagic zones (Ward and Wetzel 1980) and in unshaded areas of streams (Ward 1985; Duncan and Blinn 1989).

Photoinhibition in benthic freshwater communities generally becomes less important as these communities age (Hill and Boston 1991) and biofilm thickness increases (Dodds et al. 1999). Interestingly, Dodds et al. (1999) found that biofilms with unicellular cyanobacteria at 2–4 mm depth, or biofilms densely dominated by *Phormidium*, exhibited less photoinhibition than biofilms dominated by green algae or diatoms. Protection is provided by the biofilm matrix surrounding the microbial community and the shading induced by the layering of cells. Cell layering may be influenced by trichome motility within the mat to reduce UV damage (Bebout and Garcia-Pichel 1995; Chaps. 18 and 19).

9.2.3 Temperature

Cyanobacteria are important members of benthic communities across the complete temperature range where phototrophs occur. This is made clear for low temperature environments by Wynn-Williams (2000) and in Chap. 14, and for high temperature environments in some of the most detailed accounts of cyanobacteria in any type of community (Castenholz 1969, 1973; Ward and Castenholz 2000; Chap. 3). Although cyanobacteria often dominate streams and lakes in polar regions (Vincent 2004), they also tend to become more abundant in the benthic communities of temperate streams and lakes exposed to higher water temperatures. High cyanobacteria abundance with increased temperature has been shown in experimental systems (Cairns 1956; Patrick et al. 1969; Wilde and Tilly 1981), downstream of geothermal springs (Kullberg 1971; Lamberti and Resh 1985), and below anthropogenic thermal discharges (Hickman 1974; Patrick 1974). The mechanisms that allow cyanobacteria to dominate communities across such a wide

range of temperatures occur at the autecological (metabolic rate, cell composition and tolerances) and the population levels (growth rates, temperature optima: DeNicola 1996).

Cyanobacteria from polar freshwater systems and from cold mountain streams have been shown to exhibit optimum growth and photosynthesis between 15°C and 35°C (Mosser and Brock 1976; Tang et al. 1997), although possible interactions of other factors were not fully investigated in these studies. Other factors such as resistance to desiccation may be more important in favouring cyanobacterial dominance in these systems (Vincent and Howard-Williams 1986). In contrast, cyanobacteria from hydrothermal springs appear to be well-adapted to high water temperatures, with photosynthetic efficiencies that peak close to the upper limit for algal growth (Brock 1967). Thus, high-temperature conditions lend a competitive advantage for cyanobacteria relative to other algal taxa (DeNicola 1996; Tilman and Kiesling 1984).

Nitrogen fixation by diazotroph cyanobacteria appears to have a direct relationship to temperature across the range commonly observed in freshwater ecosystems. For instance, a hot spring study by Stewart (1970) reported an optimum temperature for nitrogen fixation (by Mastigocladus) at around 43°C. Others have measured linear increases in N_2 fixation rates up to 25°C for benthic cyanobacteria from an oligotrophic lake (Reuter et al. 1983). Marcarelli and Wurtsbaugh (2006) found that a 5°C increase can increase benthic N_2 fixation rates twofold in streams.

9.2.4 Nutrients

The freshwater benthic habitat lies at an important intersection of resource availability. Shallow environments such as wetlands, streams, and lake benthos are intimately linked with nutrient sources from surrounding terrestrial environments. Wetland and lake sediments and water resurfacing from stream hyporheic zones or groundwater can also supply nutrients to benthic autotrophs (Henry and Fisher 2003; Hagerthey and Kerfoot 2005). Nutrient availability is determined by the physical and chemical phenomena that control nutrient flux from these environments (Cotner et al. 2009). Nitrogen availability, in particular, is also influenced by diverse transformations that can occur in benthic environments (Bowden 1987).

Benthic freshwater habitats with low nutrient availability and/or low N:P supply ratios may favour N_2-fixing cyanobacteria (Levine and Schindler 1999; Perona et al. 1998; Douterelo et al. 2004). N_2-fixing cyanobacteria have been widely reported from benthos of lakes (Reuter et al. 1983; Hagerthey and Kerfoot 2005), streams (Ward 1985; Flecker 1996; Grimm and Petrone 1997), and wetlands (Rejmánková and Komárková 2000; Mayer and Galatowitsch 2001), and spatial and temporal variability in N_2 fixation rates are related to nutrient availability (Peterson and Grimm 1992; Scott et al. 2007; Marcarelli and Wurtsbaugh 2009). N_2 fixation rates in benthic autotroph communities can be very high (Grimm and Petrone 1997; Higgins et al. 2001; Marcarelli et al. 2008).

In a global analysis of N and P limitation from various terrestrial and aquatic (wetlands, streams, lakes, estuaries, oceans) habitats worldwide, Elser et al. (2007) found that N and P limited primary production equivalently in most ecosystems with two notable exceptions. Nitrogen limited primary production more often than P in most marine environments, but P limited primary production more frequently than N in benthic lake environments. N_2 fixation by cyanobacteria can sometimes rapidly alleviate N limitation of benthic autotroph communities (Scott et al. 2007), and lake sediments can be a major source of NH_4-N (Schindler et al. 1987; Findlay et al. 1994). These mechanisms may explain why P limits benthic primary production more than N in lake environments (Elser et al. 2007). Interestingly, primary production in other benthic habitats, including streams, appears to be limited equally by N and P (Elser et al. 2007). However, the efficiency of cyanobacterial N_2 fixation in many benthic environments is not well understood, particularly as it relates to other N transformation processes and its potential to alleviate system-wide N limitation (Marcarelli et al. 2008; Scott and McCarthy 2010).

9.2.5 Grazing

Many benthic freshwater ecosystems are characterized by high grazing rates and strong top-down control of primary producer biomass and species composition (Feminella and Hawkins 1995; Steinman 1996). The effects of grazing on cyanobacteria are complex, because it is unclear to what extent cyanobacteria are high or low quality food resources for particular grazers. This might be influenced by differences in nutrient composition; for instance, it seems likely that N_2-fixing cyanobacteria have a higher cellular N content than other primary producers, particularly in N-limited ecosystems. Before considering benthic communities in more detail, it is worth noting the complexity made clear in Chap. 7 of the interactions between planktonic *Microcystis* and its various grazers, which shows the need for detailed field and laboratory studies before coming to firm conclusions.

However, there are examples of cyanobacterial-dominated benthic communities supporting highly diverse grazing communities (Liston and Trexler 2005). Fenchel (1998a) found that the introduction of grazers into a cyanobacterial-dominated mat decreased cyanobacterial biomass by 70% within 30 days. However, this reduction resulted from both the direct effects of grazing and also physical changes within the mat caused by grazer movement and disruption (Fenchel 1998b, c). Other studies have documented grazing of cyanobacteria in wetlands, streams, and lakes by invertebrates (Kehde and Wilhm 1972; Holomuzki and Biggs 2006) and fish (Hildebrand and Towers 1927; Power et al. 1988; Abe et al. 2006).

Despite evidence that grazers can feed on benthic freshwater cyanobacteria, there is little evidence that cyanobacteria are selected specifically. In fact, there is considerable evidence that grazing fish and invertebrates either avoid, or cannot feed effectively on, cyanobacteria (Gelwick and Matthews 1992; Rosemond 1993; Gettel et al. 2007). As grazers remove more palatable taxa such as diatoms and green algae, they may open locations for cyanobacterial colonization (Flecker and Taylor 2004; Yang et al. 2009). If they remove epiphytic diatoms growing on large colonies or filaments of cyanobacteria (Dodds and Castenholz 1987; Power et al. 1988), they may reduce competition for light and nutrients. Regardless of the mechanism, the result is that cyanobacteria often dominate the benthic periphyton assemblage in streams where there is heavy grazing pressure.

Diatoms with cyanobacterial endosymbionts (Sect. 23.2.3) may be more palatable than cyanobacteria, but their adnate growth form may also make them difficult to consume when growing on hard substrates (Hill and Knight 1987; Arango et al. 2009). In contrast, N_2-fixing epiphytic diatoms appear to be very susceptible to grazing by mat-dwelling invertebrates (Power et al. 2009).

There are several mechanisms by which cyanobacteria may deter or avoid grazers in benthic communities. Physical deterrents such as mucilage and gelatinous or prostate growth forms make it difficult for some grazers to consume and digest cells (Wellnitz and Ward 2000; Power et al. 1988; Dudley and D'Antonio 1991). Basal trichomes and other specialized growth cells may allow rapid filament regeneration following grazing (Power et al. 1988; Yang et al. 2009). Although the production of cyanotoxins has been widely documented throughout freshwater ecosystems (Sivonen 1996), their effects in benthic habitats have rarely been considered. Yet, there is evidence that increases in benthic cyanobacteria in streams can affect benthic macroinvertebrate community structure, probably directly through toxicity (Aboal et al. 2002).

9.3 Lentic Freshwater Habitats

9.3.1 Wetlands

Lentic ecosystems are widespread globally, with approximately 12.4–14.4 million km^2 of lentic ecosystem, which cover more than 10% of land area not covered by ice (Lehner and Döll 2004; Downing et al. 2006). Approximately 70% of this is considered wetland environment and the remaining 30% is comprised of natural and man-made lakes (Lehner and Döll 2004). Wetland environments are generally categorized in groups such as bogs, marshes, swamps and fens based on their topographic and hydrologic characteristics. The greatest majority of wetlands (67%) occur in boreal and tropical regions, with fewer in the temperate zone (13%), where there has been a much greater loss due to human activities (Mitsch and Gosselink 2000).

Fig. 9.2 Cyanobacterial mats growing in the Florida Everglades (Photo courtesy of Scot Hagerthy)

9.3.1.1 Tropical and Subtropical Wetlands

The shifts between wet and dry seasons found in many tropical and subtropical regions means that cyanobacteria experience cyclic water level fluctuations (Gottlieb et al. 2005, 2006). In particular, the sheltered habitats provided by emergent tropical and subtropical marshes create favourable conditions for communities that are often dominated by cyanobacteria. The examples chosen here come primarily from North and Central America and the Caribbean.

Tropical and subtropical wetlands are often characterized by high dissolved mineral concentrations and very low inorganic P availability (Rejmánková et al. 1996; Rejmánková and Komárková 2005; Reddy and Delaune 2008). Many of the cyanobacteria in these marshes are calcareous species (McCormick and O'Dell 1996; Rejmánková and Komárková 2000). When physical disturbance or grazing pressure is low, cyanobacterial mats can be highly productive, accumulating organic matter at rates greater than 2 mm year^{-1} (Fenchel 1998a). Highly productive communities can precipitate calcium carbonate, which, when combined with organic matter accretion, results in densely laminated cyanobacterial mats.

Current knowledge of benthic cyanobacteria in many tropical and subtropical wetlands, including important ones such as the Pantanal (De-Lamonica-Freire and Heckman 1996), is limited. However, more detailed information has been assembled for marshes in Florida and Belize. Several early reports indicated that communities in the Florida Everglades (Fig. 9.2) were largely dominated by *Schizothrix calcicola* and *Scytonema hofmanni* (Van Meter 1965) with dozens of other species also present (Gleason and Spackman 1974; Wood and Maynard 1974). McCormick and O'Dell (1996)

reported that over 100 cyanobacterial species occurred in the metaphyton of the Florida Everglades. But, molecular genotypic approaches such as restriction fragment length polymorphism (RFLP) have revealed a large percentage of novel sequences (Jasrotia and Ogram 2008), suggesting that phenotypic approaches may only show a fraction of the actual genetic diversity here. Other studies have focused on cyanobacterial epiphytes (Vymazal and Richardson 1995) or cyanobacteria growing on artificial substrates (McCormick et al. 1996); the last were slightly less species rich, but otherwise had a broadly similar species composition.

Cyanobacterial communities in the tropical marshes of Belize occur in very similar habitats to those in the Everglades, although these marshes are not as large and are more fragmented (Rejmánková et al. 1996). Cyanobacterial diversity and species composition in these marshes are also similar to those in the Everglades (Rejmánková and Komárková 2000). However, a more detailed account of cyanobacterial species and morphotypes has been recently published for the Belize marshes (Komárek and Komárková-Legnerová 2007; Turicchia et al. 2009), including a description of ten new species of coccoid cyanobacteria and nine new species and one entirely new genus of oscillatorialean morphotypes. Cyanobacterial diversity in Belize marshes appears to be partially correlated with the conductivity of the marsh waters, with highest diversity occurring in marshes with medium conductivity and lower diversity at high and low conductivity levels (Rejmánková et al. 2004a).

Cyanobacterial species richness, and metaphyton biomass in general, has decreased in the Everglades and Belize marshes due to P inputs from surrounding anthropogenic activities (Grimshaw et al. 1993; Rejmánková 2001). Phosphorus enrichment in Belize and Everglades marshes has increased periphyton P content (Rejmánková and Komárková 2000; McCormick et al. 2001; Gaiser et al. 2004), decreased the severity of P limitation (Newman et al. 2003; Sirova et al. 2006), increased the severity of N limitation (Inglett et al. 2004, 2009; Rejmánková and Komárková 2000; Rejmánková et al. 2004b), and changed periphyton species composition (McCormick and O'Dell 1996; Rejmánková and Komárková 2005). More specifically, P enrichment appears to displace calcium-precipitating cyanobacteria with faster growing green algae and non-calcifying cyanobacteria (Rejmánková et al. 2004b; Gaiser et al. 2004; Jasrotia and Ogram 2008). Areal metaphyton biomass and productivity also decreases in response to P enrichment, with metaphyton primary production 6–30 times less in the P rich portions of the Everglades due to displacement by macrophytes (McCormick et al. 1998). *Typha domingensis* in particular is filling the historically open-water slough habitat previously occupied by calcite-precipitating cyanobacteria in the Everglades (Fig. 9.3a; McCormick et al. 2009). Hagerthey et al. (2008) found that multiple microbial-plant community regime shifts were related to increasing P concentrations, which eventually led to the displacement of metaphyton by *Typha domingensis* (Fig. 9.3b). Cyanobacterial mats disintegrated when benthic P concentrations increased to more than 500 mg kg^{-1}. They were replaced initially by submersed stands of *Eleocharis* and then by floating stands of *Nymphaea*. *Typha* became dominant when benthic P concentrations exceed 1,250 mg kg^{-1}. The loss of cyanobacterial biodiversity in these ecosystems is caused simultaneously by the displacement of calcite-precipitating cyanobacteria by green algae and diatoms (Gaiser et al. 2006), and the loss of the metaphyton habitat due to increased aquatic macrophytes (Hagerthey et al. 2008).

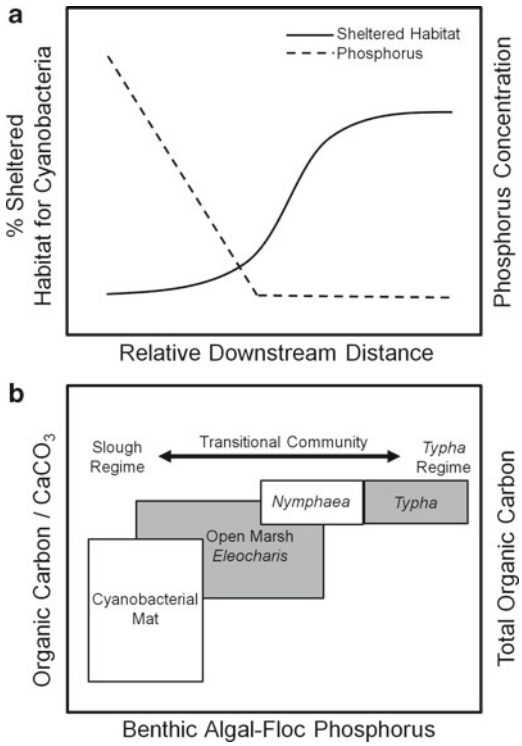

Fig. 9.3 (**a**) Spatial pattern cyanobacterial habitat and phosphorus concentrations in the Everglades relative to water inflow structures (Adapted from McCormick et al. 2009). (**b**) Sequence of regime shifts related to P concentration resulting in a displacement of cyanobacterial mats (Adapted from Hagerthey et al. 2008)

9.3.1.2 Temperate Wetlands

Temperate wetlands include riverine marshes, peatlands, rain-fed depressions such as potholes and playas and backwater swamps. Research on benthic cyanobacteria in temperate wetlands is sparse, and primarily involves benthic algal communities as a whole. Few sites have been characterized sufficiently to develop theories on the major controls on cyanobacteria in temperate wetlands. However, the existing literature identifies some common themes with other benthic environments such as water level fluctuations and nutrient availability.

Table 9.2 Distribution of world rice crop areas and percentage of different cropping practices from 2004–2006 (Adapted from International Rice Research Institute 2009; www.irri.org)

Location	Total rice area (km^2)	Percent of total rice area		Wetland rice area
		Wetland	Upland	
Africa	77,920	67.3[a]	32.6	52,440
Asia	1,350,260	93.3[b]	6.7	1,259,790
Australia	460	100[c]	0.0	460
Europe	5,810	100[c]	0.0	5,810
Latin America	53,870	53.3[d]	46.7	28,710
USA	12,830	100[c]	0.0	12,830
World	1,501,150	90.6	9.4	1,360,040

[a]Composed of 33.9% irrigated, 52.6.4% rainfed lowlands, and 13.5% deepwater crops
[b]Composed of 62.8% irrigated, 34.4% rainfed lowlands, and 2.8% deepwater crops
[c]Composed of 100% irrigated crops
[d]Composed of 90.6% irrigated and 5.4% rainfed lowland crops

Cyanobacteria occur in the natural and restored prairie marshes of north-central North America, which include the prairie potholes and delta marshes between closely associated lakes. Similar to constructed wetlands, diatoms with cyanobacterial endosymbionts (*Rhopolodia gibba* and *Epithemia* sp.) can dominate periphyton in some of these restored wetlands (Mayer and Galatowitsch 2001), but only a few studies (e.g McDougal et al. 1997) have characterized the taxonomic composition of benthic autotroph communities in detail.

Although temperate wetlands have frequently been drained, many are now being restored or replaced with created and constructed wetland ecosystems. Constructed wetlands are most often built to serve as water quality improvement structures (Kadlec and Wallace 2009), but they also provide important wildlife habitat which makes them popular for restoration or wildlife habitat improvement (Fleming-Singer and Horne 2006). Cyanobacteria can be abundant in constructed wetlands, particularly in locations where water chemistry has been modified greatly by N transformation processes (Fleming-Singer and Horne 2006; Scott et al. 2005). Benthic autotroph biomass is generally greatest near the wetland inflow where nutrient concentrations are high, but biomass can decrease along the flow path of the water as nutrient availability decreases (Wu and Mitsch 1998). Interestingly, but not surprisingly, the removal efficiencies of N and P in constructed wetlands can differ substantially due in part to the potential fates of the nutrients in wetland environments. Phosphorus removal from wetland waters relies on transient storage in sediments, but N removal can be permanent through microbial transformations such as denitrification (Scott et al. 2008). As a result, not only can nutrient availability decrease in general along the flow path of water in constructed wetlands, but the ratio of N:P can also decrease as N is removed permanently and often more efficiently than P. Thus, N_2-fixing cyanobacteria can dominate benthic autotroph communities in downstream areas within constructed wetlands (Scott et al. 2005). Similar responses have been reported in other hydrologically-altered aquatic ecosystems (Vis et al. 2008).

9.3.1.3 Rice Fields

Wetland rice fields are the most dominant human-managed wetland habitat worldwide and comprise as much as 20% of the remaining wetland area globally (Table 9.2). More than 90% of rice production occurs in wetland environments. Wetland rice fields comprise a major habitat for cyanobacteria in many locations worldwide (Table 9.2). Similar environmental constraints to those in natural marshes determine the occurrence and ecological function of cyanobacteria in wetland rice fields. However, perhaps more than any other factor, human activity influences the ecology of wetland rice field cyanobacteria (Whitton 2000).

N_2 fixation by heterocystous cyanobacteria may be important to wetland rice ecosystems as a biological fertilizer (Kundu and Ladha 1995; Dobermann and White 1999; Ladha and Reddy 2003). Early research on N_2 fixation in wetland rice fields focused on quantifying its sources. Roger and Ladha (1992) found that the N contribution from cyanobacteria (0–80 kg N ha^{-1} crop) was often higher, but more variable, than fixed N inputs from heterotrophic bacteria in the rice rhizosphere (1–7 kg N ha^{-1} crop) or heterotrophic bacteria living freely in the soil (1–31 kg N ha^{-1} crop). The effectiveness of cyanobacterial N_2 fixation as a N source to rice relies on the efficiency of N transfer between the cyanobacterial community and a rice plant (Roger 1995). The transfer of fixed N to the root zone can be slower than cyanobacterial N_2 fixation rates, causing ammonium accumulation within the cyanobacterial-periphtyton matrix and/or water column, and a subsequent decline in N_2 fixation (Simpson et al. 1994; Irisarri et al. 2001). This problem could be overcome by timing the growth of N_2-fixers with the increased demand for N by rice in late stages of plant development. Greenhouse experiments revealed that inoculating a mixed culture of *Aulosira fertilissima*, *Nostoc muscorum* and *Anabaena* sp. into a rice-soil system when rice plants

were in early- to mid- growth stage enhanced rice grain yield (Ghosh and Saha 1993). However, this management practice is not practical in large scale systems, because there is not an efficient way to inoculate large areas with live cyanobacterial cultures. Rather, the use of cyanobacterial N_2 fixation as a N source to rice has focused on culturing cyanobacteria alone, or in symbiotic association with *Azolla* (Chap. 24) during early flood stages of rice production.

N_2-fixing cyanobacteria can provide 19–28 kg N ha^{-1} annually in some rice cultivation systems and reduce the need for urea fertilizer by 25–35% (Hashem 2001). Many other studies have documented similar N inputs from cyanobacteria to rice production systems (Kannaiyan et al. 1997; Kennedy and Islam 2001; Norman et al. 2003). Choudhury and Kennedy (2004) reported N inputs in rice systems by Azolla were two times greater than N inputs from benthic cyanobacterial assemblages. As a result, rice grain in fields containing Azolla achieved 50% greater yields than rice in fields without Azolla. This enhanced N input and rice yield was attributed to more reliable management of fixed N inputs through Azolla rather than benthic cyanobacteria alone. Furthermore, fixed N inputs from cyanobacteria may only provide a fraction of the N demand in rice production systems where a high grain yield is demanded (Norman et al. 2003). Nevertheless large areas for rice cultivation, such as in Bangladesh, are still maintained without added fertilizer and this is where it is most important to optimize cyanobacterial N_2 fixation. Elsewhere cyanobacterial N_2 fixation can reduce fertilizer demand and the use of cyanobacteria as a biofertilizer is increasing at a rate to merit more concentrated research (Irisarri et al. 2007). Cyanobacteria also increase dissolved organic carbon and O_2 concentrations in rice floodwaters, which can influence rates of nutrient cycling and enhance rice yield (Mandal et al. 1999).

9.3.2 Lake Benthos

The importance of benthic primary producers, although often ignored by limnologists (Vadeboncoeur et al. 2002) is primarily dictated by light availability. Most lakes are small (Downing et al. 2006) and benthic processes may be especially important here because of a high degree of interaction between benthic and pelagic zones of lakes with small volume and size (Fee 1979). The area of a lake where light reaches the bottom is controlled primarily by lake size, morphometry and water clarity (Vadeboncoeur et al. 2008). Dissolved organic matter (Bowling et al. 1986), particulate material including phytoplankton (Robarts and Zohary 1984) and sediment (Nolen et al. 1985) all influence water clarity. Increased phytoplankton abundance can suppress benthic photoautotrophs via shading (Vadeboncoeur et al. 2001), so benthic cyanobacteria may have a larger role in oligotrophic lakes with high light penetration than in eutrophic lakes. However, it should be pointed out that the benthos of eutrophic lakes can have large *Microcystis* populations in winter, which persist in the dark until spring and in some cases for longer (Chap. 7). It seems possible that this applies to other taxa, but this topic requires further study.

The distribution and role of benthic cyanobacteria may also be controlled by nutrient availability, including competition with phytoplankton for nutrients, ability to obtain N via N_2 fixation and the ability to utilize N released from sediment pools (Hansson 1988). The roles of substrate stability and grazing for benthic cyanobacteria may also be important, but have received little study in lakes. Shallow lake zones are characterized by a diversity of benthic cyanobacterial habitats, including sediments (which can be rich in nutrients or organic matter), rocks, wood and macrophytes. Periphyton communities on these different substrates can exhibit very different compositions and productivities (Vadeboncoeur et al. 2006). Cyanobacteria may be important members of communities on one substrate, but not another, and differences among substrates may be an important source of spatial heterogeneity.

9.3.2.1 Groundwater and Spring-Fed Lakes

Among temperate lakes, benthic algae have been especially well studied in groundwater and spring-fed lakes, where benthic water inflows can alter the nutrient concentrations of the entire lake and strongly influence benthic dynamics. For example, lakes of the upper Midwestern United States receive variable amounts of groundwater input depending on their position in the landscape, and the degree of groundwater input can control within-lake characteristics like water chemistry and the distribution of primary production (Kratz et al. 1997). Seepage fluxes of groundwater have been linked to benthic periphyton dynamics in Sparkling Lake in the Northern Highland Lake District of Wisconsin (Hagerthey and Kerfoot 1998, 2005). Areas of high groundwater input are associated with high concentrations of soluble reactive P and low NH_4-N concentrations compared to areas of low groundwater input (Hagerthey and Kerfoot 1998), resulting in low N:P at high groundwater input sites. Benthic periphyton communities are less diverse at high groundwater input sites (with low N:P) and are dominated by diatoms and *Oscillatoria limosa*. In contrast, low groundwater discharge sites with high N:P are dominated by diatoms and coccoid cyanobacteria (*Aphanocapsa* sp., *Aphanothece* sp. and *Chroococcus* sp.; Hagerthey and Kerfoot 2005). Whether or not these differences are related to the ability to fix N_2 is still unknown.

Spring-fed ponds and lakes may have very high throughput rates of flow and in some ways act as hybrids of lakes and streams, with important effects on benthic processes. For example, Mare's Egg Spring, Oregon, which had a lake turnover time of only 0.3 days (Dodds and Castenholz 1988a, b), water originating at 4.5°C, and very low nitrate concentrations,

lacked any significant phytoplankton population (Dodds and Castenholz 1988a, b). Nevertheless the system was named for its large populations of *Nostoc pruniforme*, which could reach a large size and grow for many years. For example, a single 2.6 kg wet weight colony was estimated to be between 9.4 and 14.6 years old, and an average sized colony is usually between 3.4 and 5.1 years old (Dodds and Castenholz 1987; Dodds et al. 1995). It was concluded that high light levels were a major factor in the success of this species (Dodds and Castenholz 1987, 1988b). Limnocorral experiments showed that reduced flow led to an increase in phytoplankton density and a reduced growth rate of *N. pruniforme* (Dodds and Castenholz 1988a). Grazing snails did not consume *N. pruniforme*, but did remove epiphytic diatoms from their surface, which appears to increase the growth rate of the cyanobacterial colonies (Dodds and Castenholz 1987). Although *N. pruniforme* is abundant and can fix N_2 at rates typical of other lakes, N_2 fixation contributes less than 4% of the annual N budget to this pond (Dodds and Castenholz 1988b).

9.3.2.2 Subalpine Lakes

Subalpine lakes in the Rocky Mountains, western USA, are distributed widely and characterized by low nutrient concentrations due to nutrient-poor bedrock geologies. However, these lakes experience slight P elevation due to the history of volcanic activity (Fisher 2006) which causes low N:P ratios in the water; in general anthropogenic activity is low enough to have little impact. As a result, these lakes tend to be oligotrophic with very high water clarity, and much of the benthos receives light sufficient for periphyton. The proliferation of N_2-fixing benthic cyanobacteria in these lakes appears to result from the combination of high light and low N:P. Common epilithic genera in such lakes from northern California to central Idaho include *Nostoc, Calothrix* and *Tolypothrix*, as well as non-heterocystous genera such as *Chroococcus*, *Gloeocapsa*, *Lyngbya* and *Oscillatoria* (Loeb and Reuter 1981; Reuter and Axler 1992; Marcarelli and Wurtsbaugh 2009), while the epipelon tends to be colonized by unicellular colonial and non-heterocystous filamentous cyanobacteria (Reuter and Axler 1992).

Epilithic communities in several subalpine lakes were shown to exhibit a very low uptake capacity for dissolved NO_3-N and NH_4-N, suggesting that they are not adapted to compete with phytoplankton for water column N and instead rely on N_2 fixation to meet N requirements (Reuter et al. 1985, 1986; Reuter and Axler 1992). For example, in Lake Tahoe, epilithic N_2 fixation peaks during the summer with warm water temperatures (Reuter et al. 1983) and contributes a substantial portion of the N input into epilithic communities (Reuter et al. 1986). In contrast, epipelic periphyton (including non-heterocystous cyanobacteria) have access to a large N pool stored in lake sediments, and show no adaptations for living under N-deficient conditions (Reuter and Axler 1992).

Although benthic N_2 fixation is not a large contribution to the overall N budget of subalpine lakes (<1% in Lake Tahoe: Reuter et al. (1983); <5% in Bull Trout Lake: Marcarelli and Wurtsbaugh (2009)), it can be an important driver of benthic dynamics, and a seasonally important N source. For example, in Bull Trout Lake, N contributions via N_2 fixation in lake benthic zones can exceed hydrologic inputs of dissolved N during late summer months, when flows are generally low (Marcarelli and Wurtsbaugh 2009). Macrophytes can also be abundant in some subalpine lakes and high rates of N_2 fixation have been found on epiphytic communities (Marcarelli and Wurtsbaugh 2009). High rates of epiphytic N fixation activity have also been reported in other lakes (Moeller and Roskoski 1978; Corkran and Wickstrom 1987; Wickstrom and Corkran 1997), and in one situation the cyanobacteria *Gloeotrichia* sp. was identified as the primary N_2-fixer (Finke and Seeley 1978).

9.3.2.3 Tropical and Subtropical Lakes

Lakes are widely distributed in most tropical areas (Lehner and Döll 2004; Downing et al. 2006), and support benthic algal and cyanobacterial communities in habitats similar to lakes in other regions of the world. Cyanobacteria comprise as much as 30–50% of epiphyton, epipelon, and metaphyton biomass in subtropical Lake Okeechobee, Florida, USA (Havens et al. 1999b; Carrick and Steinman 2001). The relative proportion of benthic autotroph and phytoplankton biomass in Lake Okeechobee is controlled by large gradients in light climate and turbulent wave action, but periphyton species composition is influenced mainly by nutrient availability (Carrick and Steinman 2001). Periphyton communities here are often N-limited or co-limited by both N and P (Havens et al. 1996, 1999a; Rodusky et al. 2001).

Some of the Rift Valley lakes in tropical Africa are relatively unproductive due to underlying bedrock geologies that have led to historically low-nutrient habitats (Bootsma and Hecky 1993). As a result, some of these lakes support large areas of phototrophic epilithic communities (Higgins et al. 2003; O'Reilly 2006). The epilithon in these lakes is generally dominated by N_2-fixing cyanobacteria such as *Calothrix* and diatoms including *Rhopalodia* with its N_2-fixing cyanobacteria-related internal bodies (Higgins et al. 2003). N_2 fixation by epilithic cyanobacteria in Lake Malawi may contribute 30% of the annual N input to the lake (Higgins et al. 2001; Chap. 23, Sect. 23.2.3). Epilithon communities in these lakes are strongly grazed by fish and microcrustacea, and support a very high biological diversity in the food web (O'Reilly 2006). In fact, epilithon primary producers and their grazers interact on multiple levels. For example, epilithon biomass in Lake Tanganyika appears to be influenced simultaneously by top-down (grazer) and bottom-up (nutrient) controls (McIntyre et al. 2006). Nutrient regeneration by cichlid fishes accounted for 46% of the N and 48% of the P supporting epilithon growth in nearby Lake Malawi. Thus, the ecological functioning

of epilithon communities in the Rift Valley lakes are strongly tied to both fixed N inputs from cyanobacteria and interactions with secondary consumers.

N_2 fixation is also an important mechanism favouring benthic cyanobacteria in tropical floodplain lakes of the Amazon. Seasonal floodwaters are colonized by floating grass-meadows, which can support dense epiphyton communities that are largely comprised of cyanobacteria (Engle and Melack 1990). Steep nutrient gradients develop in these lakes along a perpendicular axis from the river inputs, with highest nutrient availability near the river and lowest concentrations at locations furthest from the river (Engle and Melack 1993). Epiphyton communities within the rhizospheres of the floating meadows are composed largely of cyanobacteria (Putz 1997) that fix N_2 in response to decreased inorganic N availability far away from river inflows (Doyle and Fisher 1994). N_2 fixation by epiphytes in floating meadows is generally light-dependent (Enrich-Prast and Esteves 1998) and rates can differ between different floating grass species (Kern and Darwich 2003). Epiphyton N_2 fixation appears to offset N limitation in these communities and primary production by epiphytes exceeds the annual production by phytoplankton in these lakes (Doyle and Fisher 1994).

9.4 Lotic Freshwaters

Lotic freshwater sites can be classified usefully according to the type of vegetation in their catchment (Table 9.3). Cyanobacteria are widely distributed here. For example, they comprised 24% of all taxa described in North American streams surveyed between 10°N and 73°N, which include tropical, tundra, forested, desert and coastal plain regions (Sheath and Cole 1992). Cyanobacteria can have an important role in the benthic community in streams, particularly in extreme (e.g., the geothermal and polar regions mentioned above) or specialized situations (e.g., high Zn environments: Whitton et al. 1981) and when they contribute to the N cycle via N_2-fixation (Marcarelli et al. 2008). In all streams, their ecological role is constrained and controlled most closely by a combination of hydrologic conditions and disturbance, light, water temperature, nutrient availability and grazing.

Table 9.3 Distribution of lotic habitats draining landscapes globally (Adapted from Dodds 1997)

	Runoff in streams and rivers	
Landscape type	(km^3 $year^{-1}$)	(%)
Coniferous forest	14,663	29.8
Grasslands	13,709	27.9
Temperate forest	9,438	19.2
Polar (Tundra/Ice)	6,073	12.3
Cultivated land	4,377	8.9
Desert/shrub	909	1.8

Our focus here is primarily on stream benthos. Also, because streams in extreme environments are treated in Chaps. 3 (hot springs) and 13 (polar regions), here we will focus on temperate streams, specifically some well-studied examples from deciduous forest, coniferous forest, alpine and desert streams. We will also briefly discuss the role of cyanobacteria in tropical streams, where they have been mostly studied as part of the food base for a diverse community of grazers.

9.4.1 Temperate Streams

9.4.1.1 Deciduous Forest Streams

Deciduous forest streams are widely distributed throughout the temperate zone, and have been well-studied throughout North America, Europe, and parts of Asia. They are typified by a dense canopy cover that limits light availability to the stream bed during much of the growing season and supplies seasonal pulses of organic matter input via leaf litter. Because of these large allochthonous inputs, it is often assumed that algae and cyanobacteria are of secondary importance in these streams, yet several genera of cyanobacteria are commonly found and they show important seasonal variations and community interactions.

Walker Branch has been a long-term study site of the Oak Ridge National Laboratory, and is one of the best studied forested streams in North America (Elwood and Nelson 1972; Mulholland 1992, 2004). Because of dense canopy cover, light limits algal biomass (Rosemond 1994; Rosemond et al. 2000) and primary production (Hill et al. 2001; Roberts et al. 2007) during the summer. However, during the winter and spring prior to leaf-out, light penetration to the stream bed is high, primary production peaks (Roberts et al. 2007) and algal biomass can be limited by availability of both N and P (Elwood et al. 1981; Rosemond et al. 1993). Longitudinal studies in this stream (Mulholland and Rosemond 1992) showed that depleted nutrient concentrations favour *Chamaesiphon investiens*. Perhaps because of strong light limitation, N_2-fixing cyanobacteria are rare in Walker Branch, although they have been observed during spring, particularly during a P enrichment experiment when *Oscillatoria* sp. and *Nostoc* sp. became obvious in the community (Elwood et al. 1981).

Although light and nutrients are important influences, standing stocks of periphyton in Walker Branch are most closely controlled by grazing snails (Steinman 1992; Mulholland and Rosemond 1992; Rosemond 1994). Snail grazing rates can equal the entire periphyton production rate of the stream (Elwood and Nelson 1972). Grazing maintains communities that are low growing and grazer resistant, such as *Chamaesiphon investiens* and some green algae (Mulholland and Rosemond 1992). When grazers were removed, diatoms overgrew these grazer-resistant communities, suggesting that cyanobacteria dominate the periphyton assemblage in this

stream because of their resistance to grazing (Rosemond 1993; Rosemond et al. 1993). Indeed, when grazing, irradiance, and nutrient supply are manipulated in tandem, grazing was the most important factor maintaining periphyton community composition and favouring cyanobacteria, with irradiance and nutrient supply acting as secondary controls (Rosemond 1993).

9.4.1.2 Coniferous Forest Streams

In contrast to deciduous forests, coniferous forests, which often predominate at high altitudes and latitudes, do not form entirely closed canopies and therefore permit higher light penetration to the streams. Local variation in light penetration can be caused by riparian vegetation structure, landscape geomorphology including the presence of lakes, and disturbance history. The role of cyanobacteria in subalpine coniferous forest streams has been particularly well studied in the Rocky Mountains of North America. Similar factors to those described for subalpine lakes (Sect. 9.3.2.2) are important here, such as low N availability relative to P. Cyanobacteria have been widely reported in streams throughout the region (Horne and Carmiggelt 1975; Ward 1985; Arango et al. 2009), including *Calothrix*, *Anabaena* and *Nostoc*. Diatoms belonging to the Rhopalodiales with endophytic cyanobacteria are also widespread in the epilithon and as epiphytes and can be important N_2-fixers (Marcarelli and Wurtsbaugh 2006; Arango et al. 2009; Power et al. 2009).

In forested streams in Oregon, *Nostoc parmelioides* is limited to sites where the canopy cover has been altered by logging (Ward 1985). The highest densities were found in streams draining forests which had regrown from clear-cutting 10–20 years previously. *N. parmelioides* has a unique mutualistic relationship with a dipteran (*Cricotopus* sp.) which colonizes *N. parmelioides* colonies as a larva by boring into the colony and forming a tunnel to consume the colony from the inside. One *Cricotopus* individual lives in a colony for both its larval and pupal stages; the emergence of the pupal state is synchronized with hormogonia formation by *N. parmelioides*. When pupae emerge, the hormogonia are dispersed and the *N. parmelioides* colony decomposes (Brock 1960; Ward et al. 1985; Dodds and Marra 1989). This midge subsists only on the cyanobacterium and apparently has little trouble assimilating this material, perhaps because of gut adaptations (Ward et al. 1985). When colonized, *N. parmelioides* colonies change shape from globose to ear-shaped (Fig. 9.1), and ear-shaped colonies may be capable of higher maximum photosynthetic rates than globose colonies (Ward et al. 1985, but see Dodds 1989). This may be because ear-shaped colonies extend into regions of higher current velocity away from the stream bottom, hence enhancing nutrient diffusion rates (Dodds 1989).

Like many other glacially-influenced montane regions, watersheds of the Sawtooth Mountains of central Idaho, USA, are characterized by a high frequency of lakes interspersed with short distances of streams. This stream-lake landscape leads to a series of predictable differences in stream periphyton communities above and below lakes, with consequences for cyanobacteria and N_2 fixation. Lakes alter outlet streams by increasing summer water temperatures (Marcarelli and Wurtsbaugh 2007), decreasing concentrations of dissolved inorganic N and P, increasing organic N and P (Brown et al. 2008), decreasing the magnitude of spring floods (Arp et al. 2006), and increasing the stability of benthic substrates relative to lake inlet streams (Arp et al. 2007; Myers et al. 2007). As a result, overall periphyton standing stocks tend to be larger in lake outlets than inlets (Myers et al. 2007; Marcarelli and Wurtsbaugh 2007), and cyanobacteria, particularly N_2-fixing *Calothrix* sp. and *Anabaena* sp., and diatoms with cyanobacterial endosymbionts (*Epithemia sorex, E. adnata* and *Rhopalodia gibba)*, are abundant in lake outlets, while rare in inlets (Marcarelli and Wurtsbaugh 2006; Baker et al. 2009). A stream-side experiment showed results which correspond with the distribution found in the field (Marcarelli and Wurtsbaugh 2006). Altering N and P concentrations and increasing water temperature could increase N_2 fixation rates via both physiological stimulation (short-term increases in N_2 fixation with 5–10°C rise in temperature) and altered community composition (increased diazotrophs). N_2-fixation by cyanobacteria may alter N uptake patterns in outlet streams. Low nitrate uptake rates are typical of algal patches with abundant N_2-fixers (Baker et al. 2009), and N_2-fixers are associated with stream stretches where nitrate uptake is undetectable (Arp and Baker 2007; Marcarelli et al. 2008).

9.4.1.3 Alpine Streams

High altitude streams present environments where light intensity is often high, temperatures very low and typically nutrient availability also very low. As a result of these harsh conditions, periphyton communities tend to have low diversity, and cyanobacterial distribution may be limited (Hieber et al. 2001; Rott et al. 2006). Stream characteristics are often closely influenced by the presence of an upstream glacier, which controls water temperatures, water chemistry, and disturbance regime (Hieber et al. 2002). Hieber et al. (2001) reported that *Chamaesiphon* sp. was characteristic of glacial-fed sites. The presence of lakes in some watersheds below glaciers is a secondary control on stream characteristics, with lake buffering seasonal fluctuations leading to more stable flows at sites downstream of the lake outlets (Hieber et al. 2002); these sites are characterized by Oscillatoriales, especially *Phormidum* sp., *Oscillatoria* sp., and *Lyngbya* sp. (Hieber et al. 2001).

9.4.1.4 Desert Stream: Sycamore Creek

One of the most thoroughly-studied stream ecosystems in North America is Sycamore Creek in the Sonoran desert (Fisher 1986; Grimm 1994). Warm stream temperature, high insolation and limited N availability all interact to

favour cyanobacteria here and cyanobacterial mats including *Calothrix* sp. and *Anabaena* sp. often cover large portions of the stream bottom (Grimm and Petrone 1997). Like many other streams in western USA, algal biomass is N-limited, and the degree of N limitation varies with the length of time since the last large flood (Grimm and Fisher 1986; Peterson and Grimm 1992). At certain times of year, N_2 fixation by cyanobacteria can account for 85% of the benthic N flux (Grimm and Petrone 1997), with N_2 fixation rates that are among the highest measured in any stream (Marcarelli et al. 2008) and rival those in eutrophic lakes and rice paddies. The temporal dynamics of the cyanobacterial communities are controlled by intense and unpredictable flash floods that scour the stream bottom and reset community dynamics, while the spatial dynamics are controlled by high exchange rates between the hyporheic, parafluvial and riparian zones (Fisher et al. 1998).

The post-flood succession starts with diatoms, then filamentous green algae and finally cyanobacterial mats (Fisher et al. 1982). This pattern can be related to N availability, as floods usually lead to a N pulse, thus reducing N-limiting conditions for a brief time (Grimm and Fisher 1992). At early successional stages, algal standing stocks are low and rely on nutrients from the water column and recycled from the sediments. As chlorophytes join the community and begin to form mats, reduced diffusion through the mats leads to decreased nutrient uptake from the environment and strong N limitation of this algal community, thus favouring colonization by cyanobacteria (Peterson and Grimm 1992). However, the trajectory of this succession is controlled by the season (Grimm and Petrone 1997), the magnitude and degree of scour generated by the flood (Grimm and Fisher 1989; Peterson et al. 1994) and the time interval between floods (Grimm and Fisher 1989). Cyanobacterial mats are more easily scoured by high flow events than diatom communities (Grimm and Fisher 1989). Therefore, the effects of floods on cyanobacteria will depend on time since last flood (Peterson et al. 1994). During the winter when temperature and light intensity are low, cyanobacteria are rare even long after the last flood (Grimm and Petrone 1997).

The distribution of cyanobacteria in Sycamore Creek also varies spatially due to hydrologic exchange between the surface stream and hyporheic zone. The hyporheic zone in Sycamore Creek is oxic and supports high rates of coupled decomposition and nitrification (Jones et al. 1995), which results in higher concentrations of nitrate at upwelling zones where hyporheic water enters the stream, compared to downwelling zones where stream water enters the hyporheic zone (Valett et al. 1994; Henry and Fisher 2003). The same pattern happens as water moves in and out of sandbars that make up the parafluvial zone (Fisher et al. 1998). As a result, N_2-fixing cyanobacteria are more abundant at downwelling zones and along upstream sandbar edges due to decreased N availability, and less abundant at upwelling zones and along downstream sandbar edges (Grimm and Petrone 1997; Henry and Fisher 2003).

9.4.2 Tropical Streams

Benthic algal dynamics in tropical streams are controlled by similar factors to temperate streams, such as flow, light, nutrient supply and grazing. In some, but not all, cases tropical streams may differ markedly in flow, or even alternate between wet and dry states, associated with seasonal rainfall (Flecker 1992). During the wet season when flows are high, the stream bed is frequently disturbed and turbidity is high; little is known about benthic periphyton during this time other than some floristic lists for waterfalls. During the dry period flows are low, temperature is warm, water clarity is high and the bed is dominated by attached periphyton and overlying organic detritus and associated epipelon (Power 1984; Pringle and Hamazaki 1998; Flecker and Taylor 2004). Small streams can support a high diversity of vertebrates and large invertebrates that feed on benthic algae and detritus, including fish (Power 1990; Flecker 1992; Pringle and Hamazaki 1997), amphibians (Flecker et al. 1999; Ranvestel et al. 2004; Whiles et al. 2006) and decapod shrimp (Pringle 1996; Pringle and Hamazaki 1998). Many stream studies have focused on the effects of these unique animals on tropical stream structure and function, so most of what is known about cyanobacteria in tropical streams relates to top-down effects.

One of the best studied tropical streams is Rio Las Marias, a 4th-order stream of the Orinoco River drainage in the Andean piedmont of Venezuela (Flecker 1992, 1996). During the dry season, this stream has high light availability and attached algae show evidence of N-limitation (Flecker et al. 2002). Grazing fish are diverse, and include tetras, long-whiskered catfish, and armored catfish (Flecker 1996). A number of exclosure studies have demonstrated that several of these fish species act as ecosystem engineers through their benthic feeding habits, creating "feeding scars" or areas cleared of algae and detritus (Flecker 1996; Flecker and Taylor 2004); at high fish densities they can clear stream bottoms almost completely and strikingly reduce rates of primary production (Flecker and Taylor 2004; Taylor et al. 2006). These feeding scars are often colonized by cyanobacteria, including the N_2-fixers *Calothrix* and *Anabaena* (Flecker 1996; Flecker et al. 2002; Flecker and Taylor 2004). Nutrient and grazing experiments showed that *Anabaena* responded most strongly to grazing pressure when N was enriched, although it is unclear whether this was an artifact of the short experimental duration (Flecker et al. 2002). Nevertheless, top-down control of periphyton community

composition (including cyanobacteria) was stronger than any bottom-up effect of N addition (Flecker et al. 2002). Therefore, grazing by fish on benthic algal communities could have important influence on nutrient inputs, uptake and demand through mediating the presence or absence of cyanobacteria.

Similar grazing effects have been studied in tropical streams of Costa Rica and Panama, where grazers include omnivorous fish, shrimp and amphibians (Pringle and Hamazaki 1998; Whiles et al. 2006). Studies comparing fish and shrimp grazing in Costa Rican streams have demonstrated that fishes have stronger effects on benthic algal community composition than shrimps, and that fish grazing can significantly shift benthic algal assemblages towards dominance by cyanobacteria, especially *Lyngbya* (Pringle and Hamazaki 1997, 1998). Because *Lyngbya* is very resistant to scouring in these streams, fish can mediate the resistance and resilience of the benthic algal community through their grazing activity (Pringle and Hamazaki 1997). In contrast to fish grazers, which avoid grazing on or facilitate increases in cyanobacteria, grazing tadpoles in these streams may reduce cyanobacteria (Connelly et al. 2008). Experimental exclosures and natural removal of grazing tadpoles have shown a shift in the algal assemblage from small, adnate diatoms to large, upright diatoms and cyanobacteria (Ranvestel et al. 2004; Connelly et al. 2008). In these Central American streams, amphibians have declined precipitously over the past two decades, experiencing local extinctions (Whiles et al. 2006), which may in turn have major effects on the role of cyanobacteria (Connelly et al. 2008) and the effects of other grazers on algal-cyanobacterial communities including macroinvertebrates (Colón-Gaud et al. 2009).

9.5 Future Study

As evident from the previous sections, substantial progress has been made in our understanding of the ecological roles of cyanobacteria in freshwater benthic environments. However, there are many areas in which further research is justified. Two of these areas of which we are particularly interested include: (1) The role of N_2-fixing benthic cyanobacteria in alleviating N deficiency of non-N-fixing benthic microorganisms, and (2) The role of interactions between cyanobacteria and their grazers in freshwater benthic environments.

In a recent review, Marcarelli et al. (2008) found only nine published studies that quantified N_2 fixation rates in stream benthos, compared to dozens of studies focused on dissolved inorganic N uptake and other N transformations. Studies of benthic N fixation in lake littoral zones and non-human dominated wetlands are similarly rare. This is particularly striking in comparison to hundreds of published studies reporting the role of N fixation in pelagic freshwater and marine regions (Howarth et al. 1988). Clearly, more work is needed on the magnitude and importance of N fixation in benthic freshwater environments and the role of this process in freshwater ecosystem dynamics. Moreover, N inputs from cyanobacteria can support the growth and metabolism of other microorganisms that do not fix N. The planktonic cyanobacterium *Trichodesmium* in the Gulf of Mexico immediately releases more than 50% of fixed N, which can be rapidly incorporated by other planktonic microorganism (Mulholland et al. 2004, 2006). Although Finlay et al. (2011) have suggested that similar transfers occur in freshwater benthic environments, none have been demonstrated clearly. More research is needed to understand the ecosystem-scale importance of benthic N_2 fixation by cyanobacteria.

Grazing effects on cyanobacteria in freshwater benthos is also somewhat contradictory and unclear. There is evidence of high direct consumption of cyanobacteria by certain grazers in some benthic freshwater ecosystems (e.g., tropical wetlands) but not in others (e.g., most streams). It is unclear what mediates the strength of grazing pressure on cyanobacteria in these different ecosystems, and whether it is more closely related to characteristics of the cyanobacteria or characteristics of the grazers. Cyanobacteria palatability may be linked to nutrient availability; high nutrient availability may increase cyanobacterial nutrient content, making cells more nutritious for grazers, but may also allow cyanobacteria to manufacture defense mechanisms like toxins and mucilage (Anderson et al. 2002). Diazotroph cyanobacteria may be more nutritious for grazers due to high cellular N content, but it is evident from the literature reviewed above that not all benthic freshwater grazers can feed on them. For example, fish avoid grazing on cyanobacteria in tropical streams of Central America, while amphibians do not (Pringle and Hamazaki 1997; Connelly et al. 2008). Diatoms with cyanobacterial endosymbionts may be more palatable for consumers than cyanobacteria, although they may also be resistant to grazers because of their adnate growth form (see Arango et al. 2009; Power et al. 2009). The ability of grazers to feed on cyanobacteria is important because grazing is a pathway by which fixed N may be incorporated into freshwater food webs and ecosystems, and grazers have the ability to move nutrients between benthic and pelagic habitats and control ecosystem nutrient stoichiometry via grazing and excretion (Vanni 2002; McIntyre et al. 2006).

9.6 Conclusions

Cyanobacteria are abundant in freshwater benthic environments globally. This chapter has outlined the major constraints on cyanobacteria in these environments and summarized research on freshwater benthic habitats that has contributed broadly to our understanding of cyanobacterial ecology. The

comparative analysis provided in the chapter indicates that many common factors such as flow, light, nutrients, and grazing determine cyanobacterial abundance and diversity across different benthic habitats. However, our understanding of how cyanobacteria function in freshwater environments is still growing. Freshwater habitats are threatened worldwide by a variety of anthropogenic activities that may alter environmental conditions (Malmqvist and Rundle 2002). We expect that the diversity and function of cyanobacteria in these habitats will change as environmental conditions change. For example, increasing water demand by humans may favour desiccation-resistant cyanobacteria over less resistant chlorophytes (Benenati et al. 1998). Human activity is likely to modify all of the environmental controls discussed in this chapter and non-linear interactions between environmental factors and ecological communities may make predicting the effects of environmental change on cyanobacteria difficult or impossible. However, research that integrates across environmental factors and habitats to understand the ecological context of cyanobacterial responses to disturbance can provide insight that may direct management and mitigation activities.

References

Abe SI, Kiso K, Katano O, Yamamoto S, Nagumo T, Tanaka J (2006) Impacts of differential consumption by the grazing fish, *Plecoglossus altivelis*, on the benthic algal composition in the Chikuma River, Japan. Phycol Res 54:94–98

Aboal M, Puig MA, Mateo P, Perona E (2002) Implications of cyanophyte toxicity on biological monitoring of calcareous streams in north-east Spain. J Appl Phycol 14:49–56

Anderson DM, Gilbert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries 25:704–726

Arango CP, Riley LA, Tank JL, Hall RO (2009) Herbivory by an invasive snail increases nitrogen fixation in a nitrogen-limited stream. Can J Fish Aquat Sci 66:1309–1317

Arp CD, Baker MA (2007) Discontinuities in stream nutrient uptake below lakes in mountain drainage networks. Limnol Oceanogr 52:1978–1990

Arp CD, Gooseff MN, Baker MA, Wurtsbaugh WA (2006) Surface-water hydrodynamics and regimes of a small mountain stream-lake ecosystem. J Hydrol 329:500–513

Arp CD, Schmidt JC, Baker MA, Myers AK (2007) Stream geomorphology in a mountain lake district: hydraulic geometry, sediment sources and sinks, and downstream lake effects. Earth Surf Proces Land 32:525–543

Baker MA, de Guzman G, Ostermiller JD (2009) Differences in nitrate uptake among benthic algal assemblages in a mountain stream. J N Am Benthol Soc 28:24–33

Bebout BM, Garcia-Pichel F (1995) UV B-induced vertical migrations of cyanobacteria in a microbial mat. Appl Environ Microbiol 61:4215–4222

Benenati PL, Shannon JP, Blinn DW (1998) Dessication and recolonization of phytobenthos in a regulated desert river: Colorodo River at Lees Ferry, Arizona, USA. Regul River 14:519–532

Biggs BJF, Goring DG, Nikora VI (1998) Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. J Phycol 34:598–607

Blenkinsopp SA, Lock MA (1994) The impact of storm-flow on river biofilm architecture. J Phycol 30:807–818

Bootsma HA, Hecky RE (1993) Conservation of the African Great Lakes: a limnological perspective. Conserv Biol 7:644–656

Borchardt MA (1996) Nutrients. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic, San Diego, pp 183–227, 753 pp

Bourassa N, Cattaneo A (2000) Responses of a lake outlet community to light and nutrient manipulation: effects on periphyton and invertebrate biomass and composition. Freshw Biol 44:629–639

Bowden WB (1987) The biogeochemistry of nitrogen in freshwater wetlands. Biogeochemistry 4:313–348

Bowling LC, Steane MS, Tyler PA (1986) The spectral distribution and attenuation of underwater irradiance in Tasmanian inland waters. Freshw Biol 16:313–335

Brock EM (1960) Mutualism between the midge Cricotopus and the alga Nostoc. Ecology 41:474–483

Brock TD (1967) Relationship between standing crop and primary productivity along a hot spring thermal gradient. Ecology 48:566–571

Brown PD, Wurtsbaugh WA, Nydick KR (2008) Lakes and forests as determinants of downstream nutrient concentrations in small mountain watersheds. Arct Antarct Alp Res 40:462–469

Cairns J Jr (1956) Effects of increased temperatures on aquatic organisms. Ind Waste 1:150–152

Carpenter SR, Lodge DM (1986) Effects of submersed macrophytes on ecosystem processes. Aquat Bot 26:341–370

Carrick HJ, Steinman AD (2001) Variation in periphyton biomass and species composition in Lake Okeechobee, Florida (USA): distribution of algal guilds along environmental gradients. Arch Hydrobiol 152:411–438

Castenholz RW (1969) Thermophilic blue-green algae and the thermal environment. Bacteriol Rev 33:476–504

Castenholz RW (1973) Ecology of blue-green algae in hot springs. In: Carr NG, Whitton BA (eds) The biology of blue-green algae. Blackwell, Oxford, pp 379–414, 688 pp

Choudhury ATMA, Kennedy IR (2004) Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biol Fertil Soil 39:219–227

Colón-Gaud C, Whiles MR, Kilham SS, Lips KR, Pringle CM, Connelly S, Peterson SD (2009) Assessing ecological responses to catastrophic amphibian declines: patterns of macroinvertebrate production and food web structure in upland Panamanian streams. Limnol Oceanogr 54:331–343

Connelly S, Pringle CM, Bixby RJ, Brenes R, Whiles MR, Lips KR, Kilham S, Huryn AD (2008) Changes in stream primary producer communities resulting from large-scale catastrophic amphibian declines: can small-scale experiments predict effects of tadpole loss? Ecosystems 11:1262–1276

Corkran JL, Wickstrom CE (1987) Diel patterns of nitrogenase activity associated with macrophytes in a eutrophic lake. Aquat Bot 28:341–352

Cotner JB, Kenning J, Scott JT (2009) The microbial role in littoral zone biogeochemical processes: why Wetzel was right. Verh Int Ver Limnol 30:981–984

Davies-Colley RJ, Hickey CW, Quinn JM, Ryan PA (1992) Effects of clay discharges on streams. 1. Optical properties and epilithon. Hydrobiologia 248:215–234

De-Lamonica-Freire EM, Heckman CW (1996) The seasonal succession of biotic communities in wetlands of the tropical wet and dry climate zone: III. The algal communities in the Pantanal of Matto Grasso, Brazil, with a comprehensive list of the known species and revision of two desmid taxa. Int Rev Ges Hydrobiol 81:253–280

DeNicola DM (1996) Periphyton responses to temperature at different ecological levels. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic, San Diego, pp 149–181, 753 pp

Dobermann A, White PF (1999) Strategies for nutrient management in irrigated and rainfed lowland rice systems. Nutr Cycl Agroecosyst 53:1–18

Dodds WK (1989) Photosynthesis of two morphologies of *Nostoc parmelioides* (Cyanobacteria) as related to current velocities and diffusion patterns. J Phycol 25:258–262

Dodds WK (1997) Distribution of runoff and rivers related to vegetative characteristics, latitude, and slope: a global perspective. J N Am Benthol Soc 16:162–168

Dodds WK, Castenholz RW (1987) Effects of grazing and light on the growth of *Nostoc pruniforme* (Cyanobacteria). Br Phycol J 23:219–227

Dodds WK, Castenholz RW (1988a) The biological effects of nitrate fertilization and water replacement in an oligotrophic cold water pond. Hydrobiologia 162:141–146

Dodds WK, Castenholz RW (1988b) The nitrogen budget of an oligotrophic cold water pond. Arch Hydrobiol Suppl 79:343–362

Dodds WK, Marra JL (1989) Behaviors of the midge, Cricotopus (Diptera: Chironomidae) related to mutualism with *Nostoc parmelioides* (Cyanobacteria). Aquat Insect 11:201–208

Dodds WK, Gudder DA, Mollenhauer D (1995) The ecology of *Nostoc*. J Phycol 31:2–18

Dodds WK, Biggs BJF, Lowe RL (1999) Photosynthesis-irradiance patterns in benthic microalgae: variations as a function of assemblage thickness and community structure. J Phycol 35:42–53

Douglas B (1958) The ecology of the attached diatoms and other algae in a small stony stream. J Ecol 46:295–322

Douterelo I, Perona E, Mateo P (2004) Use of cyanobacteria to assess water quality in running waters. Environ Pollut 127:377–384

Downing JA, Prairie YT, Cole JJ, Duarte CM, Tranvik LJ, Striegel RG, McDowell WH, Kortelainen P, Caraco NF, Melack JM, Middelburg JJ (2006) Global abundance and size distribution of lakes, ponds, and impoundments. Limnol Oceanogr 51:2388–2397

Doyle RD, Fisher TR (1994) Nitrogen fixation by periphyton and plankton on the Amazon floodplain at Lake Calado. Biogeochemistry 26:41–66

Dudley TL, D'Antonio CM (1991) The effects of substrate texture, grazing, and disturbance on macroalgal establishment in streams. Ecology 72:297–309

Duncan SW, Blinn DW (1989) Importance of physical variables on the seasonal dynamics of epilithic algae in a highly shaded canyon stream. J Phycol 25:455–461

Durako MJ, Medlyn RA, Moffler MD (1982) Particulate matter resuspension via metabolically produced gas bubbles from benthic estuarine microalgal communities. Limnol Oceanogr 27:752–756

Elser JJ, Bracken MES, Cleland EA, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine, and terrestrial ecosystems. Ecol Lett 10:1135–1142

Elwood JW, Nelson DL (1972) Periphyton production and grazing rates in a stream measured with a 32P material balance method. Oikos 23:295–303

Elwood JW, Newbold JD, Trimble AF, Stark RW (1981) The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62:146–158

Engle DL, Melack JM (1990) Floating meadow epiphyton: biological and chemical features of epiphytic material in an Amazon floodplain lake. Freshw Biol 22:479–494

Engle DL, Melack JM (1993) Consequences of riverine flooding for seston and the periphyton of floating meadows in an Amazon floodplain lake. Limnol Oceanogr 38:1500–1520

Enrich-Prast A, Esteves FA (1998) Diurnal variation of rates of denitrification and nitrogen fixation of periphyton associated with Oryza glumaepatula (Steud) in an Amazonian Lake. Hydrobiologia 368:189–192

Fee EJ (1979) A relation between lake morphometry and primary productivity and its use in interpreting whole-lake eutrophication experiments. Limnol Oceanogr 24:401–416

Feminella JW, Hawkins CP (1995) Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. J N Am Benthol Soc 14:465–509

Fenchel T (1998a) Formation of laminated cyanobacterial mats in the absence of benthic fauna. Aquat Microb Ecol 14:235–240

Fenchel T (1998b) Artificial cyanobacterial mats: structure and composition of the biota. Aquat Microb Ecol 14:241–251

Fenchel T (1998c) Artificial cyanobacterial mats: cycling of C, O, and S. Aquat Microb Ecol 14:253–259

Finlay JC, Hood JM, Limm MP, Power ME, Schade JD, Welter JR (2011) Light-mediate thresholds in stream-water nutrient composition in a river network. Ecology 92:140–150

Findlay DL, Hecky RE, Hendzel LL, Stainton MP, Regehr GW (1994) Relationship between N_2 fixation and heterocyst abundance and its relevance to the nitrogen budget in Lake 227. Can J Fish Aquat Sci 51:2254–2266

Finke LR, Seeley HW (1978) Nitrogen fixation (acetylene reduction) by epiphytes of freshwater macrophytes. Appl Environ Microbiol 36:129–138

Fisher SG (1986) Structure and dynamics of desert streams. In: Whitford WG (ed) Pattern and process in desert ecosystems. University of New Mexico Press, Albuquerque, pp 119–139, 139 pp

Fisher SG (2006) Stream ecosystems of the western United States. In: Cushing CE, Cummins KW, Minshall GW (eds) River and stream ecosystems of the world. University of California Press, Berkeley, pp 61–87, 817 pp

Fisher SG, Gray LJ, Grimm DB, Busch DE (1982) Temporal succession in a desert stream ecosystem following flash flooding. Ecol Monogr 52:93–110

Fisher SG, Grimm NB, Martí E, Holmes RM, Jones JB (1998) Material spiraling in stream corridors: a telescoping ecosystem model. Ecosystems 1:19–34

Flecker AS (1992) Fish trophic guilds and the structure of a tropical stream: weak direct vs. strong indirect effects. Ecology 73:927–940

Flecker AS (1996) Ecosystem engineering by a dominant detritivore in a diverse tropical stream. Ecology 77:1845–1854

Flecker AS, Taylor BW (2004) Tropical fishes as biological bulldozers: density effects on resource heterogeneity and species diversity. Ecology 85:2267–2278

Flecker AS, Feifarek BP, Taylor BW (1999) Ecosystem engineering by a tropical tadpole: density-dependent effects on habitat structure and larval growth rates. Copeia 1999:495–500

Flecker AS, Taylor BW, Bernhardt ES, Hood JM, Cornwell WK, Cassatt SR, Vanni MJ, Altman NS (2002) Interactions between herbivorous fishes and limiting nutrients in a tropical stream ecosystem. Ecology 83:1831–1844

Fleming-Singer MS, Horne AJ (2006) Balancing wildlife needs and nitrate removal in constructed wetlands: the case of the Irvin Ranch Water District's San Joaquin Wildlife Sanctuary. Ecol Eng 26:147–166

Francoeur SN, Biggs BJF (2006) Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. Hydrobiologia 561:59–69

Gaiser EE, Scinto LJ, Richards JH, Jayachandran K, Childers DL, Trexler JC, Jones RD (2004) Phosphorus in periphyton mats provides the best metric for detecting low-level P enrichment in an oligotrophic wetland. Water Res 38:507–516

Gaiser EE, Childers DL, Jones RD, Richards JH, Scinto LJ, Trexler JC (2006) Periphyton response to eutrophication in the Florida Everglades: cross-systems patterns of structural and compositional change. Limnol Oceanogr 51:617–630

Gelwick FP, Matthews WJ (1992) Effects of an algivorous minnow on temperate stream ecosystem properties. Ecology 73:1630–1645

Gettel GM, Giblin AE, Howarth RM (2007) The effects of grazing by the snail, Limnaea elodes, on benthic N_2 fixation and primary production in oligotrophic, arctic lakes. Limnol Oceanogr 52:2398–2409

Ghosh TK, Saha KC (1993) Effects of inoculation with N2-fixing cyanobacteria on the nitrogenase activity of soil and rhizosphere of wetland rice. Biol Fertil Soil 16:16–20

Gleason PJ, Spackman W (1974) Calcareous periphyton and water chemistry in the Everglades. In: Gleason PJ (ed) Environments of South Florida: present and past. Miami Geological Society, Miami, pp 146–181

Gottlieb A, Richards J, Gaiser E (2005) Effects of desiccation duration on the community structure and nutrient retention of short and long-hydroperiod Everglades periphyton mats. Aquat Bot 82:99–112

Gottlieb A, Richards JH, Gaiser EE (2006) Comparative study of periphyton community structure in long and short-hydroperiod Everglades marshes. Hydrobiologia 569:195–207

Grimm NB (1994) Disturbance, succession and ecosystem processes in streams: a case study from the desert. In: Giller PS, Hildrew AG, Rafaelli DG (eds) Aquatic ecology: scale, pattern and process. Blackwell Scientific, Oxford, pp 93–112, 649 pp

Grimm NB, Fisher SC (1986) Nitrogen limitation in a Sonoran Desert stream. J N Am Benthol Soc 5:2–15

Grimm NB, Fisher SC (1989) Stability of periphyton and macroinvertebrates to disturbance by flash floods in a desert stream. J N Am Benthol Soc 8:293–307

Grimm NB, Fisher SC (1992) Responses of arid-land streams to changing climate. In: Firth P, Fisher SG (eds) Global climate change and freshwater ecosystems. Springer, New York, pp 211–233, 321 pp

Grimm NB, Petrone KC (1997) Nitrogen fixation in a desert stream ecosystem. Biogeochemistry 37:33–61

Grimshaw HJ, Rosen M, Swift DR, Rodberg K, Noel JM (1993) Marsh phosphorus concentrations, phosphorus content and species composition of Everglades periphyton communities. Arch Hydrobiol 128:257–276

Hagerthey SE, Kerfoot WC (1998) Groundwater flow influences the biomass and nutrient ratios of epibenthic algae in a north temperate seepage lake. Limnol Oceanogr 43:1227–1242

Hagerthey SE, Kerfoot WC (2005) Spatial variation in groundwater-related resource supply influences freshwater benthic algal assemblage composition. J N Am Benthol Soc 24:807–819

Hagerthey SE, Newman S, Rutchey K, Smith EP, Godin J (2008) Multiple regime shifts in a subtropical peatland: community-specific thresholds to eutrophication. Ecol Monogr 78:547–565

Hansson LA (1988) Effects of competitive interactions on the biomass development of planktonic and periphytic algae in lakes. Limnol Oceanogr 33:121–128

Hashem MA (2001) Problems and prospects of cyanobacterial biofertilizer for rice cultivation. Aust J Plant Physiol 28:881–888

Havens KE, East TL, Meeker RH, Davis WP, Steinman AB (1996) Phytoplakton and periphyton responses to in-situ experimental nutrient enrichment in a shallow subtropical lake. J Plankton Res 18:551–566

Havens KE, East TL, Rodusky AJ, Sharfstein B (1999a) Littoral periphyton responses to nitrogen and phosphorus: and experimental study in a subtropical lake. Aquat Bot 63:267–290

Havens KE, East TL, Hwang SJ, Rodusky AJ, Sharfstein B, Steinman AB (1999b) Algal responses to experimental nutrient addition in the littoral community of a subtropical lake. Freshw Biol 42:329–344

Henry JC, Fisher SG (2003) Spatial segregation of periphyton communities in a desert stream: causes and consequences for N cycling. J N Am Benthol Soc 22:511–527

Hickman M (1974) Effects of the discharge of thermal effluent from a power station on Lake Wabamun, Alberta, Canada – the epipelic and episamic algal communities. Hydrobologia 45:199–215

Hieber M, Robinson CT, Rushforth SR, Uehlinger U (2001) Algal communities associated with different alpine stream types. Arct Antarct Alp Res 33:447–456

Hieber M, Robinson CT, Uehlinger U, Ward JV (2002) Are Alpine lake outlets less harsh than other alpine streams. Arch Hydrobiol 154:199–223

Higgins SN, Hecky RE, Taylor WD (2001) Epilithic nitrogen fixation in the rocky littoral zones of Lake Malawi, Africa. Limnol Oceanogr 46:976–982

Higgins SN, Kling HJ, Hecky RE, Taylor WD, Bootsma HA (2003) The community composition, distribution, and nutrient status of epilithic periphyton at five rocky littoral zones sites in Lake Malawi, Africa. J Gt Lakes Res 29:181–189

Hildebrand SF, Towers IL (1927) Food of trout in Fish Lake, Utah. Ecology 8:389–397

Hill WR, Boston HL (1991) Community development alters photosynthesis-irradiance relations in stream periphyton. Limnol Oceanogr 36:1375–1389

Hill WR, Knight AW (1987) Experimental analysis of the grazing interaction between and mayfly and stream algae. Ecology 68:1955–1965

Hill WR, Ryon MG, Schilling EM (1995) Light limitation in a stream ecosystem: responses by primary producers and consumers. Ecology 76:1297–1309

Hill WR, Mulholland PJ, Marzolf ER (2001) Stream ecosystem responses to forest leaf emergence in spring. Ecology 82:2306–2319

Holomuzki JR, Biggs BJF (2006) Food limitation affects algivory and grazer performance for New Zealand stream macroinvertebrates. Hydrobiologia 561:83–94

Horne AJ (1975) Algal nitrogen fixation in California streams: diel cycles and nocturnal fixation. Freshw Biol 5:471–477

Horne AJ, Carmiggelt CJW (1975) Algal nitrogen fixation in Californian streams: seasonal cycles. Freshw Biol 5:461–470

Howarth RW, Marino R, Lane J, Cole JJ (1988) Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. Limnol Oceanogr 33:669–687

Inglett PW, Reddy KR, McCormick PV (2004) Periphyton chemistry and nitrogenase activity in a northern Everglades ecosystem. Biogeochemistry 67:213–233

Inglett PW, D'Angelo EM, Reddy KR, McCormick PV, Hagerthy SE (2009) Periphyton nitrogenase activity as an indicator of wetland eutrophication: spatial patterns and responses to phosphorus dosing in a northern Everglades ecosystem. Wetl Ecol Manage 17:131–144

Irisarri P, Gonnet S, Monza J (2001) Cyanobacteria in Uruguayan rice fields: diversity, nitrogen fixing ability, and tolerance to herbicide and combined nitrogen. J Biotechnol 91:95–103

Irisarri P, Gonnet S, Deambrosi E, Monza J (2007) Cyanobacterial inoculation and nitrogen fertilization in rice. World J Microbiol Technol 23:237–242

Jasrotia P, Ogram A (2008) Diversity of nifH genotypes in floating periphyton mats along a nutrient gradient in the Florida Everglades. Curr Microbiol 56:563–568

Jones JB, Fisher SG, Grimm NB (1995) Nitrification in the hyporheic zone of a desert stream ecosystem. J N Am Benthol Soc 14:249–258

Jørgensen BB, Cohen Y, Des Marais DJ (1987) Photosynthetic action spectra and adaptation to spectral light distribution in a benthic cyanobacterial mat. Appl Environ Microbiol 53:879–886

Kadlec RH, Wallace SD (2009) Treatment wetlands, 2nd edn. CRC Press, Boca Raton, 1025 pp

Kannaiyan S, Aruna SJ, Kumari S, Hall DO (1997) Immobilized cyanobacteria as a biofertilizer for rice crops. J Appl Phycol 9:167–174

Kehde PM, Wilhm JL (1972) The effects of grazing by snails on community structure of periphyton in laboratory streams. Am Midl Nat 87:8–24

Kennedy IR, Islam N (2001) The current and potential contribution of asymbiotic nitrogen fixation to nitrogen requirements on farms: a review. Aust J Exp Agric 41:447–457

Kern J, Darwich A (2003) The role of periphytic N_2 fixation for stands of macrophytes in the whitewater floodplain (varzea). Amazoniana 17:361–375

Komárek J, Komárková-Legnerová J (2007) Taxonomic evaluation of the cyanobacterial microflora from alkaline marshes of Northern Belize. 1. Phenotypic diversity of coccoid morphotypes. Nova Hedwig 84:65–111

Kratz TK, Webster KE, Bowser CJ, Macnuson JJ, Benson BJ (1997) The influence of landscape position on lakes in northern Wisconsin. Freshw Biol 37:209–217

Kullberg RG (1971) Algal distribution in six thermal spring effluents. Trans Am Microsc Soc 90:412–434

Kundu DK, Ladha JK (1995) Enhancing soil nitrogen use and biological nitrogen fixation in wetland rice. Exp Agric 31:261–277

Ladha JK, Reddy PM (2003) Nitrogen fixation in rice systems: state of knowledge and future prospects. Plant Soil 19:151–167

Lamberti GA, Resh VH (1985) Distribution of benthic algae and macroinvertebrates along a thermal stream gradient. Hydrobiologia 128:13–21

Lehner B, Döll P (2004) Development and validation of a global database of lakes, reservoirs and wetlands. J Hydrol 296:1–22

Levine SN, Schindler DW (1999) Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. Can J Fish Aquat Sci 56:451–466

Liston SE, Trexler JC (2005) Spatiotemporal patterns in community structure of macroinvertebrates inhabiting calcareous periphyton mats. J N Am Benthol Soc 24:832–844

Livingstone D, Pentecost A, Whitton BA (1984) Diel variations in nitrogen and carbon dioxide fixation by the blue-green alga *Rivularia* in an upland stream. Phycologia 23:125–133

Loeb SL, Reuter JE (1981) The epilithic periphyton community: a five-lake comparative study of community productivity, nitrogen metabolism and depth-distribution of standing crop. Verh Int Ver Limnol 21:346–352

Luttenton MR, Rada RG (1986) Effects of disturbance on epiphytic community architecture. J Phycol 22:320–326

Malmqvist B, Rundle S (2002) Threats to the running water ecosystems of the world. Environ Conserv 29:134–153

Mandal B, Vlek BLG, Mandal LN (1999) Beneficial effects of blue-green algae and *Azolla*, excluding supplying nitrogen, on wetland rice fields: a review. Biol Fertil Soil 28:329–342

Marcarelli AM, Wurtsbaugh WA (2006) Temperature and nutrient supply interact to control nitrogen fixation in oligotrophic streams: an experimental examination. Limnol Oceanogr 51:2278–2289

Marcarelli AM, Wurtsbaugh WA (2007) Effects of upstream lakes on nutrient limitation on periphytic biomass and nitrogen fixation in oligotrophic, subalpine streams. Freshw Biol 52:2211–2225

Marcarelli AM, Wurtsbaugh WA (2009) Nitrogen fixation varies spatially and seasonally in linked stream-lake ecosystems. Biogeochemistry 94:95–110

Marcarelli AM, Baker MA, Wurtsbaugh WA (2008) Is in-stream N_2 fixation an important N source for benthic communities and stream ecosystems? J N Am Benthol Soc 27:186–211

Mayer PM, Galatowitsch SM (2001) Assessing integrity of restored prairie wetlands from species production-diversity relationships. Hydrobiologia 443:177–185

McCormick PV, O'Dell MB (1996) Quantifying periphyton responses to phosphorus in the Florida Everglades: a synoptic experimental approach. J N Am Benthol Soc 15:450–468

McCormick PV, Rawlick PS, Lurding K, Smith EP, Sklar FH (1996) Periphtyon-water quality relationships along a nutrient-gradient in the northern Florida Everglades. J N Am Benthol Soc 15:433–449

McCormick PV, Shuford RBE, Backus JG, Kennedy WC (1998) Spatial and seasonal patterns of periphyton biomass and productivity in the northern Everglades, Florida, USA. Hydrobiologia 362:185–208

McCormick PV, O'Dell MB, Shuford RBE, Backus JG, Kennedy WC (2001) Periphyton responses to experimental phosphorus enrichment in a subtropical wetland. Aquat Bot 71:119–131

McCormick PV, Newman S, Vilchek LW (2009) Landscape responses to wetland eutrophication: loss of slough habitat in the Florida Everglades, USA. Hydrobiologia 621:105–114

McDougal RL, Goldsborough LG, Hann BJ (1997) Responses of a prairie wetland to press and pulse additions of inorganic nitrogen and phosphorus: production by planktonic and benthic algae. Arch Hydrobiol 140:145–167

McIntire CD (1966) Some effects of current velocity on periphyton communities in laboratory streams. Hydrobiologia 27:559–570

McIntyre PB, Michel E, Olsgard M (2006) Top-down and bottom-up controls on periphyton biomass and productivity in Lake Tanganyika. Limnol Oceanogr 51:1514–1523

Mitsch WJ, Gosselink JG (2000) Wetlands, 3rd edn. Wiley, New York, 920 pp

Moeller RE, Roskoski JP (1978) Nitrogen-fixation in the littoral benthos of an oligotrophic lake. Hydrobiologia 60:13–16

Mosser JL, Brock TD (1976) Temperature optima for algae inhabiting cold mountain stream. Arct Antarct Alp Res 8:111–114

Mulholland PJ (1992) Regulation of nutrient concentrations in a temperate forest stream: roles of upland, riparian, and instream processes. Limnol Oceanogr 37:1512–1526

Mulholland PJ (2004) The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch Watershed. Biogeochemistry 70:403–426

Mulholland PJ, Rosemond AD (1992) Periphyton response to longitudinal nutrient depletion in a woodland stream: evidence of upstream-downstream linkage. J N Am Benthol Soc 11:405–419

Mulholland MR, Bronk DA, Capone DG (2004) Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by Trichodesmium IMS101. Aquat Microb Ecol 37:85–94

Mulholland MR, Bernhardt PW, Heil CA, Bronk DA, O'Neil JM (2006) Nitrogen fixation and the release of fixed nitrogen by *Trichodesmium* spp. in the Gulf of Mexico. Limnol Oceanogr 51:1762–1776

Myers AK, Marcarelli AM, Arp CD, Baker MA, Wurtsbaugh WA (2007) Disruptions of stream sediment size and stability by lakes in mountain watersheds: potential effects on periphyton biomass. J N Am Benthol Soc 26:390–400

Neumann AC, Gebelein CD, Scoffin TP (1970) The composition, structure and erodability of subtidal mats, Abaco, Bahamas. J Sediment Petrol 40:274–297

Newman S, McCormick PV, Backus JG (2003) Phosphatase activity as an early warning indicator of wetland eutrophication: problems and prospects. J Appl Phycol 15:45–59

Nolen SL, Wilhm J, Howick G (1985) Factors influencing inorganic turbidity in a Great Plains reservoir. Hydrobiologia 123:109–117

Norman RJ, Wilson CE, Slaton NA (2003) Soil fertilization and rice nutrition in US mechanized rice culture. In: Smith CW, Dilday RH (eds) Rice: origin, history, technology, and production. Wiley Sciences, Hoboken, pp 331–411

O'Reilly C (2006) Seasonal dynamics of periphyton in a large tropical lake. Hydrobiologia 553:293–301

Patrick R (1974) Effects of abnormal temperatures on algal communities. In: Gibbons JW, Sharitz RR (eds) Thermal ecology: proceedings of a symposium held at Augusta, Georgia, 3–5 May 1973. US Atomic Energy Commission, Oak Ridge, pp 335–349, 670 pp

Patrick R, Crum B, Coles J (1969) Temperature and manganese as determining factors in the presence of diatom or blue-green algal floras in streams. Proc Natl Acad Sci USA 64:472–478

Perona E, Bonilla I, Mateo P (1998) Epilithic cyanobacteria communities and water quality: an alternative tool for monitoring eutrophication in the Alberche River (Spain). J Appl Phycol 10:183–191

Peterson CG (1996) Responses of benthic algal communities to natural physical disturbance. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic, San Diego, pp 375–402, 753 pp

Peterson CG, Grimm NB (1992) Temporal variation in enrichment effects during periphyton succession in a nitrogen-limited desert stream ecosystem. J N Am Benthol Soc 11:20–36

Peterson CG, Hoagland KD, Stevenson RJ (1990) Timing of wave disturbance and the resistance and recovery of a freshwater epilithic microalgal community. J N Am Benthol Soc 9:54–67

Peterson CG, Weibel AC, Grimm NB, Fisher SG (1994) Mechanisms of benthic algal recovery following spates: comparison of simulated and natural events. Oecologia 98:280–290

Poff NL, Voelz NJ, Ward JV, Lee RE (1990) Algal colonization under four experimentally-controlled current regimes in a high mountain stream. J N Am Benthol Soc 9:303–318

Power ME (1984) The importance of sediment in the grazing ecology and size class interactions of an armored catfish, Ancistrus spinosus. Environ Biol Fish 10:173–181

Power ME (1990) Resource enhancement by indirect effects of grazers: armored catfish, algae, and sediment. Ecology 71:897–904

Power ME, Stewart AJ, Matthews WJ (1988) Grazer control of algae in an Ozark mountain stream: effects of short-term exclusion. Ecology 69:1894–1898

Power M, Lowe R, Furey P, Welter J, Limm M, Finlay J, Bode C, Chang S, Goodrich M, Sculley J (2009) Algal mats and insect emergence in rivers under Mediterranean climates: toward photogrammetric surveillance. Freshw Biol 54:2101–2115

Pringle C (1996) Atyid shrimps (Decapoda: Atyidae) influence the spatial heterogeneity of algal communities over different scales in tropical montane streams, Puerto Rico. Freshw Biol 35:125–140

Pringle CM, Hamazaki T (1997) Effects of fishes on algal response to storms in a tropical stream. Ecology 78:2432–2442

Pringle CM, Hamazaki T (1998) The role of omnivory in a neotropical stream: separating diurnal and nocturnal effects. Ecology 79:269–280

Putz R (1997) Periphyton communities in Amazonian black- and whitewater habitats: community structure, biomass, and productivity. Aquat Sci 59:74–93

Ranvestel AW, Lips KR, Pringle CM, Whiles MR, Bixby RJ (2004) Neotropical tadpoles influence stream benthos: evidence for the ecological consequences of decline in amphibian populations. Freshw Biol 49:274–285

Reddy KR, DeLaune RD (2008) Biogeochemistry of wetlands: science and applications. CRC Press, Boca Raton, 774 pp

Rejmánková E (2001) Effect of experimental phosphorus enrichment on oligotrophic tropical marshes in Belize, Central America. Plant Soil 236:33–53

Rejmánková E, Komárková J (2000) A function of cyanobacterial mats in phosphorus-limited tropical wetlands. Hydrobiologia 431:135–153

Rejmánková E, Komárková J (2005) Response of cyanobacterial mats to nutrient and salinity changes. Aquat Bot 83:87–107

Rejmánková E, Pope KO, Post R, Maltby E (1996) Herbaceous wetlands of the Yucatan Peninsula: communities at extreme ends of environmental gradients. Int Rev Ges Hydrobiol 81:223–252

Rejmánková E, Komárek J, Komárková J (2004a) Cyanobacteria – a neglected component of biodiversity: patterns of species diversity of inland marshes of northern Belize (Central America). Divers Distrib 10:189–199

Rejmánková E, Komárková J, Rejmánek M (2004b) δ15N as an indicator of N_2-fixation by cyanobacterial mats in tropical marshes. Biogeochemistry 67:353–368

Reuter JE, Axler RP (1992) Physiological characteristics of inorganic nitrogen uptake by spatially separate algal communities in a nitrogen-deficient lake. Freshw Biol 27:227–236

Reuter JE, Loeb SL, Goldman CR (1983) Nitrogen fixation in periphyton of oligotrophic Lake Tahoe. In: Wetzel RG (ed) Periphyton of freshwater ecosystems. Dr W. Junk Publishers, The Hague, pp 101–109, 359 pp

Reuter JE, Loeb SL, Axler RP, Carlton RG, Goldman CR (1985) Transformations of nitrogen following an epilimnetic nitrogen fertilization in Castle Lake, CA: 1. Epilithic periphyton responses. Arch Hydrobiol 102:425–433

Reuter JE, Loeb SL, Goldman CR (1986) Inorganic nitrogen uptake by epilithic periphyton in a N-deficient lake. Limnol Oceanogr 31:149–160

Riber HH, Wetzel RG (1987) Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton. Limnol Oceanogr 32:1181–1194

Robarts RD, Zohary T (1984) Microcystic aeruginosa and underwater light attenuation in a hypereutrophic lake (Hartbeespoort Dam, South Africa). J Ecol 72:1001–1017

Roberts BJ, Mulholland PJ, Hill WR (2007) Multiple scales of temporal variability in ecosystem metabolism rates: results from 2 years of continuous monitoring in a forested headwater stream. Ecosystems 10:588–606

Robson BJ (2000) Role of residual biofilm in the recolonization of rocky intermittent streams by benthic algae. Mar Freshw Res 51:725–732

Robson BJ, Matthews TG, Lind PR, Thomas NA (2008) Pathways for algal recolonization in seasonally-flowing streams. Freshw Biol 53:2385–2401

Rodusky AJ, Steinman AD, East TL, Sharfstein B, Meeker RH (2001) Periphyton nutrient limitation and other growth-controlling factors in Lake Okeechobee, USA. Hydrobiologia 448:27–39

Roger PA (1995) Biological N_2-fixation and its management in wetland rice cultivation. Fertil Res 42:261–276

Roger PA, Ladha JK (1992) Biological N_2 fixation in wetland rice fields: estimation and contribution to nitrogen balance. Plant Soil 141:41–55

Romani AM, Sabater S (1997) Metabolism recovery of a stromatolitic biofilm after drought in a Mediterranean stream. Arch Hydrobiol 140:261–271

Rosemond AD (1993) Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia 94:585–594

Rosemond AD (1994) Multiple factors limit seasonal variation in periphyton in a forest stream. J N Am Benthol Soc 13:333–344

Rosemond AD, Mulholland PJ, Elwood JW (1993) Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74:1264–1280

Rosemond AD, Mulholland PJ, Brawley SH (2000) Seasonally shifting limitation of stream periphyton: response of algal populations and assemblage biomass and productivity to variation in light, nutrients and herbivores. Can J Fish Aquat Sci 57:66–75

Rott E, Cantonati M, Fureder L, Pfister P (2006) Benthic algae in high altitude streams of the Alps – a neglected component of the aquatic biota. Hydrobiologia 562:195–216

Schindler DW, Hesslein RH, Turner MA (1987) Exchange of nutrients between sediments and water after 15 years of experimental eutrophication. Can J Fish Aquat Sci 44:26–33

Scott JT, Doyle RD, Filstrup CT (2005) Periphyton nutrient limitation and nitrogen fixation potential along a wetland nutrient-depleation gradient. Wetlands 25:439–448

Scott JT, Doyle RD, Back JA, Dworkin SI (2007) The role of N_2 fixation in alleviating N limitation in wetland metaphyton: enzymatic, isotopic, and elemental evidence. Biogeochemistry 84:207–218

Scott JT, McCarthy MJ, Gardner WS, Doyle RD (2008) Denitrification, dissimilatory nitrate reduction to ammonium, and nitrogen fixation along a nitrate concentration gradient in a created freshwater wetland. Biogeochemistry 87:99–111

Scott JT, McCarthy MJ (2010) Nitrogen fixation may not balance the nitrogen pool of lakes over timescales relevant to eutrophication management. *Limnology and Oceanography* 55:1265–1270

Sheath RG, Cole KM (1992) Biogeography of stream macroalgae in North America. J Phycol 28:448–460

Simpson IC, Roger PA, Oficial R, Grant IF (1994) Effects of nitrogen fertilizer and pesticide management on floodwater ecology in a wetland ricefield. I. Experimental design and dynamics of photosynthetic aquatic biomass. Biol Fertil Soil 17:129–137

Sirova D, Vrba J, Rejmánková E (2006) Extracellular enzyme activities in benthic cyanobacterial mats: comparison between nutrient-enriched and control sites in marshes of northern Belize. Aquat Microb Ecol 44:11–20

Sivonen K (1996) Cyanobacterial toxins and toxin production. Phycologia 35(6 Suppl):12–25

Sperling JA, Hale GM (1973) Patterns of radiocarbon uptake by thermophilic blue-green alga under varying conditions of incubation. Limnol Oceanogr 18:658–666

Steinman AD (1992) Does an increase in irradiance influence periphyton in a heavily-grazed woodland stream? Oecologia 91:163–170

Steinman AD (1996) Effects of grazers on freshwater benthic algae. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic, San Diego, pp 341–373, 753 pp

Stevenson RJ (1996a) An introduction to algal ecology in freshwater benthic habitats. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic, San Diego, pp 3–30, 753 pp

Stevenson RJ (1996b) The stimulation and drag of current. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology: freshwater benthic ecosystems. Academic, San Diego, pp 321–340, 753 pp

Stevenson RJ, Stoermer EF (1981) Quantitative differences between benthic algal communities along a depth gradient in Lake Michigan. J Phycol 17:29–36

Stewart WDP (1970) Nitrogen fixation by blue-green algae in Yellowstone thermal areas. Phycologia 9:261–268

Tang EPY, Tremblay R, Vincent WF (1997) Cyanobacterial dominance of polar freshwater ecosystems: are high-latitude mat-formers adapted to low temperature? J Phycol 33:171–181

Taylor BW, Flecker AS, Hall RO (2006) Loss of a harvested fish species disrupts carbon flow in a diverse tropical river. Science 313:833–836

Tilman D, Kiesling RL (1984) Freshwater algal ecology: taxonomic trade-offs in the temperature dependence of nutrient competitive abilities. In: Klug MJ, Reddy CA (eds) Current perspectives in microbial ecology. American Society for Microbiology, Washington, DC, pp 314–319, 710 pp

Turicchia S, Ventura S, Komárková J, Komárek J (2009) Taxonomic evaluation of cyanobacterial microflora in alkaline marshes of northern Belize: diversity of oscillatorialean genera. Nova Hedwig 89:165–200

Uehlinger U (1991) Spatial and temporal variability of periphyton biomass in a pre-alpine river (Necker, Switzerland). Arch Hydrobiol 123:219–237

Vadeboncoeur Y, Lodge DM, Carpenter SR (2001) Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. Ecology 82:1065–1077

Vadeboncoeur Y, Vander Zanden MJ, Lodge DM (2002) Putting the lake back together: reintegrating benthic pathways into lake food web models. Bioscience 52:44–54

Vadeboncoeur Y, Kalff J, Christoffersen K, Jeppesen E (2006) Substratum as a driver of variation in periphyton chlorophyll and productivity in lakes. J N Am Benthol Soc 25:379–392

Vadeboncoeur Y, Peterson G, Vander Zanden MJ, Kalff J (2008) Benthic algal production across lake size gradients: interactions among morphometry, nutrients and light. Ecology 89:2542–2552

Valett HM, Fisher SG, Brimm NB, Camill P (1994) Vertical hydrologic exchange and ecological stability of a desert stream ecosystem. Ecology 75:548–560

Van Meter NN (1965) Some quantitative and qualitative aspects of periphyton in the Everglades. MS thesis, University of Miami, Coral Gables, FL, USA, 98 pp

Vanni MJ (2002) Nutrient cycling by animals in freshwater ecosystem. Annu Rev Ecol Syst 33:341–370

Vincent WF (2004) Microbial ecosystems of Antarctica. Cambridge University Press, Cambridge, 304 pp

Vincent WF, Howard-Williams C (1986) Antarctic stream ecosystems: physiological ecology of a blue-green algal epilithon. Freshw Biol 16:219–233

Vis S, Cattaneo A, Hudon C (2008) Shift from chlorophytes to cyanobacteria in benthic macroalgae along a gradient of nitrate depletion. J Phycol 44:38–44

Vymazal J, Richardson CJ (1995) Species composition, biomass, and nutrient content of periphyton in the Florida Everglades. J Phycol 31:343–354

Ward AK (1985) Factors affecting distribution of *Nostoc* in Cascade Mountain streams of Western Oregon, U.S.A. Verh Int Ver Limnol 22:2799–2804

Ward DM, Castenholz RW (2000) Cyanobacteria in geothermal habitats. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Springer, Dordrecht, pp 37–59, 669 pp

Ward AK, Wetzel RG (1980) Interactions of light and nitrogen source on planktonic blue-green algae. Arch Hydrobiol 90:1–25

Ward AK, Dahm CA, Cummins KW (1985) *Nostoc* (Cyanophyta) productivity in Oregon stream ecosystems: invertebrate influences and differences between morphological types. J Phycol 21:223–227

Wellnitz TA, Ward JV (2000) Herbivory and irradiance shape periphytic architecture in a Swiss alpine stream. Limnol Oceanogr 45:64–75

Whiles MR, Lips KR, Pringle CM, Kilham SS, Bixby RJ, Brenes R, Connelly S, Colón-Gaud JC, Hunte-Brown M, Huryn AD, Montgomery C, Peterson S (2006) The effects of amphibian population declines on the structure and function of neotropical stream ecosystems. Front Ecol Environ 4:27–34

Whitton BA (2000) Soils and rice fields. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 233–249, 669 pp

Whitton BA, Gale NL, Wixson BG (1981) Chemistry and plant ecology of zinc-rich wastes dominated by blue-green algae. Hydrobiologia 83:331–341

Wickstrom CE, Corkran JL (1997) Nitrogenase activities associated with macrophytes from a lacustrine and a freshwater estuarine habitat. Aquat Bot 59:157–162

Wilde EW, Tilly LJ (1981) Structural characteristics of algal communities in thermally altered artificial streams. Hydrobiologia 76:57–63

Wood EJG, Maynard NG (1974) Ecology of microalgae of the Florida Everglades. In: Gleason PJ (ed) Environments of South Florida: present and past. Miami Geological Society, Miami, pp 123–145

Wu X, Mitsch WJ (1998) Spatial and temporal patterns of algae in newly constructed freshwater wetlands. Wetlands 18:9–20

Wynn-Williams DD (2000) Cyanobacteria in deserts – life at the limit. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Springer, Dordrecht, pp 341–361, 669 pp

Yang GY, Tang T, Dudgeon D (2009) Spatial and seasonal variations in benthic algal assemblages in streams in monsoonal Hong Kong. Hydrobiologia 632:189–200

Subaerial Cyanobacteria

10

Allan Pentecost and Brian A. Whitton

Contents

A. Pentecost (✉)
Honorary Research Fellow of the Freshwater Biological Association,
Freshwater Biological Association, The Ferry Landing,
Far Sawrey, Ambleside, Cumbria LA22 0LP, UK
e-mail: allan.pentecost@kcl.ac.uk

B.A. Whitton
School of Biological and Biomedical Sciences,
Durham University, Durham DH1 3LE, UK
e-mail: b.a.whitton@durham.ac.uk

Summary

Subaerial cyanobacterial communities are conspicuous on the surfaces of many environments subject to considerable water stress, though the communities are increasingly likely to be endolithic the greater the water stress. In temperate regions the communities tend to be best developed on calcareous surfaces, especially in the case of strict epiliths. However, the contrast with non-calcareous surfaces is less obvious in the tropics, where many examples of well developed cyanobacterial communities have been reported from non-calcareous surfaces. Detailed floristic lists often include species of *Gloeocapsa*, *Pseudocapsa*, *Phormidium*, *Microcoleus*, *Tolypothrix*, *Scytonema*, *Dichothrix* and *Stigonema*, and also *Nostoc* from the more horizontal surfaces. Almost all taxa have a well developed extracellular matrix, which includes scytonemin or other coloured UV-protective pigments in all except the most shaded environments. Although the general effects of differences in environmental factors such as temperature, light, UV stress, pH, CO_2 and mineral nutrients are understood quite well, relatively little is known about the detailed responses to different combinations and periodicities of these factors.

10.1 Introduction

The first really detailed account of subaerial algae was by Petersen (1915), who actually used the term 'aerial', and defined them as algae obtaining most of their water from the atmosphere and undergoing frequent desiccation in the normal vegetative state. However, modern accounts mostly apply 'aerial' to organisms suspended in the atmosphere and use 'subaerial' for Petersen's algae. Schlichting (1975) defined subaerial algae as those in air over the surface of soil, litter or water. Although we have kept this simple definition in mind, we have extended it to include endolithic forms, many of which exist partly on, and partly inside, the surface. Topics covered in Chap. 11 (Cyanobacterial Biofilms in

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Monuments and Caves) and Chap. 12 (Semi-Arid Regions and Deserts) are considered only briefly here.

Many studies have included information on subaerial cyanobacteria, but few have sufficient detail to provide the understanding obtained for cyanobacteria in aquatic environments. We have, nevertheless, tried to assemble as much information as possible about their physical and chemical environment. Although desiccation is omitted from most definitions, intermittent drying is very important for almost all subaerial algae. Because of the impracticality for most studies of obtaining environmental data throughout the year, the simple terms mesic and xeric are used to provide a qualitative measure of relative wetness (Fletcher 1973). Mesic surfaces are those that remain damp or wet for long periods, while xeric surfaces are rarely wetted. In moist, sheltered situations such as the base of sandstone cliffs, permanently damp surfaces occur owing to capillarity or seepage. These intergrade with truly aquatic habitats, but it is useful to consider them here, since the water film is extremely thin. Schlichting (1975), who recognized the importance of the substrate in determining which species thrive, separated them into epiphytic, epiphyllous, corticolous, epizoic, lithophilous, epixylous and epimetallous. There is nowadays also much interest in cryophilic algae, although these are often immersed in the snow or ice rather than on the surface (Chap. 14).

Taxonomic identification is often a problem, because many subaerial cyanobacteria have a simple morphology, especially members of Chroococcales, a feature shared with subaerial green algae (Hoffmann 1989). Molecular studies are needed in genera such as *Gloeocapsa* to establish whether there is much more diversity than indicated by classical taxonomy. Even in the more complex forms it is not always clear the extent to which morphological diversity is environmental or genetic, though Uher (2010) has attempted to do this for *Petalonema alatum* by combining field and culture observations.

10.2 Physical Environment

10.2.1 Substratum

Subaerial surfaces provide a complex environment for colonizing microorganisms. Gorbushina (2007) reviewed the problems for microbes between initial colonization and the development of a biofilm. An important factor at the initial stage is the surface texture. A *Gloeocapsa* cell, for example, arriving on a fresh surface in a drop of rainwater or dust particle, is more likely to become entrapped and retained by a rough than a smooth substratum, thus leading to more rapid colonization. Such differences in microtopography of a subaerial surface have an important influence on colonization. Small declivities on an otherwise smooth surface can retain moisture, giving an environment conducive to growth, while

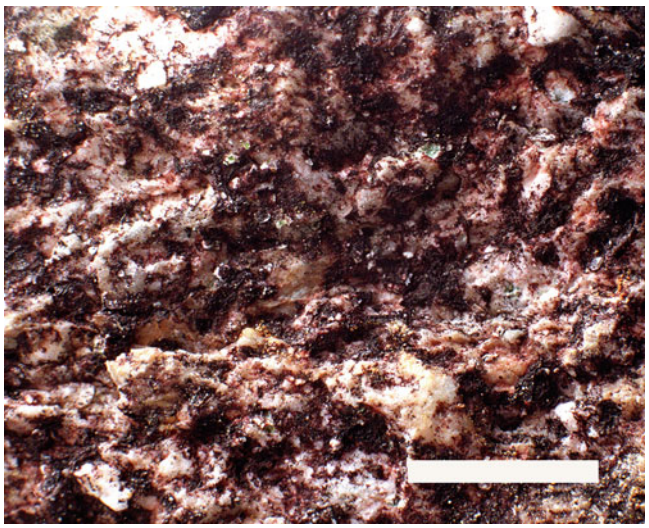

Fig. 10.1 Colonies of *Gloeocapsa sanguinea* occupying small natural depressions in the surface of a rhyolite tuff exposure near Llyn Llydaw, Snowdon, Wales. Bar = 5 mm

affording a degree of protection from grazing, wind action and other forms of abrasion and high light intensities. Such declivities may be a natural feature of the surface (Fig. 10.1). Successful colonizers at this stage are typically species forming small clumps and the clumps may be connected by fungal hyphae, which sometimes also penetrate the substrate (Gorbushina 2007). These are also likely to provide some protection for the phototrophs.

In some situations the formation of declivities is initiated or enhanced by growth of the cyanobacteria, such as the pits containing *Gloeocapsa* spp. and lichens found by Danin et al. (1982) covering limestone and dolomite surfaces in Israel. It was suggested that the 0.1–0.3 mm deep pits shielded the algae from solar radiation. Once a surface becomes colonized, growth may proceed outwards onto more exposed adjacent surfaces with water being supplied directly by precipitation, via capillarity between cells or trichomes, or diffusion from extracellular polymers such as a sheath. A cyclic pattern of colonization may be envisaged whereby surface growths become vulnerable to grazers and abrasion leading to ablation (Fig. 10.2). These protected centres of colonization may then act as refugia permitting further growth when conditions improve.

Microtopography is influenced by at least six independent factors, though not necessarily all at any one site: (i) size of the constituent mineral grains in the rock; (ii) substratum composition, which can in turn affect physical and chemical weathering; (iii) grain orientation and shape as in cleavage; (iv) nature of the grain surface that can range from smooth crystal faces to irregular, cracked or cleaved surfaces on rocks, or smooth to irregular on tree bark; (v) deep surface fractures; (vi) void spaces such as those in sandstones and

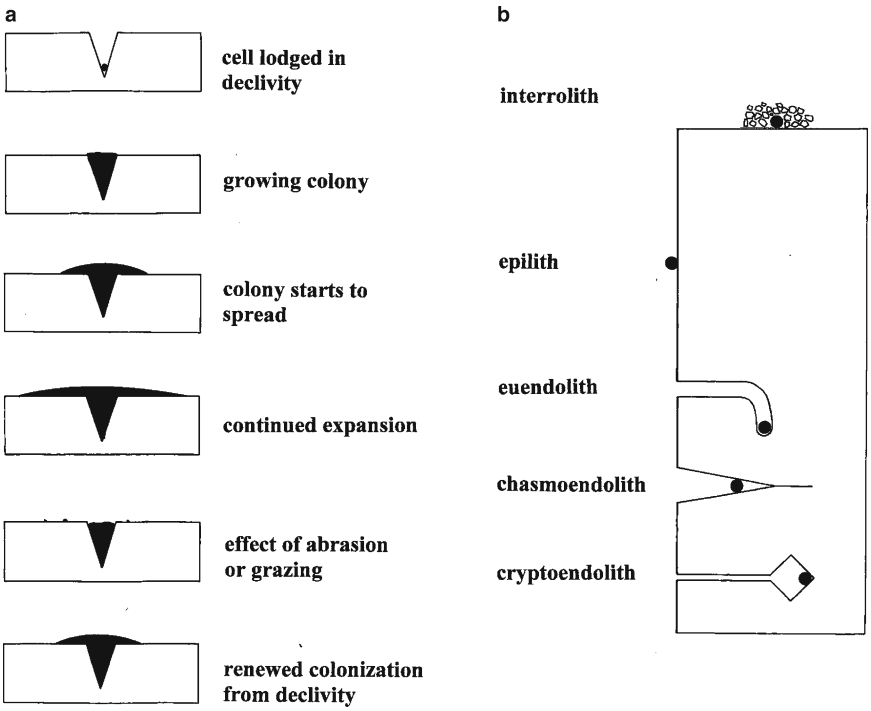

Fig. 10.2 (**a**) Colonization of a surface containing a declivity. (**b**) Relationship between subaerial cyanobacteria and substratum. (Modified from Golubic et al. 1981)

the spongy bark of trees like *Sambucus*. The last provides endolithic/endoxylic habitats and additional moisture retention. Combinations of the above factors can result in a complex surface with a wealth of microhabitats differing both physically (e.g. colour, surface energy) and chemically (mineral–water interactions). Where the surface is porous, grain size may provide a filter allowing cells below a certain size to pass into the cavities. Growth of the cells may then result in pressure forcing the grains apart.

It is often visually obvious that colonization is more extensive on rough and porous surfaces than smooth, non-porous ones (John 1988). Unfortunately, quantitative measurements in support of this are lacking, but estimates can be made to suggest why this should be so. As surface area is related to surface roughness, it can be measured using the method of fractals (Mandelbrot 1983). The relevant metric should be of the same order as the size of the colonizing unit. Taking the common genus *Gloeocapsa* to represent a 'unit' of colonization, 10 µm was chosen to represent cell diameter and estimates made of the exposed surface in a 1 × 1 cm block of weathered rock or tree bark were undertaken (Table 10.1). The values demonstrate that an apparently smooth surface such as shale along the plane of bedding had a total surface area considerably greater than a smooth plane, while across the plane there was a fivefold increase in area. Rocks with widely differing textures such as the samples of Carboniferous Limestone and granite used for the study did not differ greatly in their surface area, and neither differed greatly from the rough bark of a deciduous tree. If fractures and void spaces are included in the estimate, there would be a further increase in surface area. The measurements show the considerable area available for colonization on subaerial surfaces.

Table 10.1 Estimated total surface areas of 1 × 1 cm blocks of weathered rock and tree bark at sites in UK using a 10 µm metric (A.P., unpublished)

Substratum	Location	Surface area (cm^2)
Carboniferous Limestone	Lancashire	1.9
Granite	Eskdale, Cumbria	2.4
Shale along the bedding	Coniston, Cumbria	1.5
Shale across the bedding	Coniston, Cumbria	7.6
Bark of mature oak (*Quercus robur*)	Cumbria	4.5

10.2.2 Water Regime

There is a great diversity in the water regimes experienced by subaerial cyanobacteria. Where there is a regular annual cycle of high and negligible precipitation, the cyanobacteria may be moist or totally submerged for part of the year and entirely dry for the rest, which is usually the period with the highest irradiation. However, in a study of cyanobacterial diversity at Gimhae, Korea, it was the colder part of the year when the organisms were dry for 6–8 months (Tripathi et al. 2007). In other regions they may be wetted intermittently by precipitation for much of the year, providing

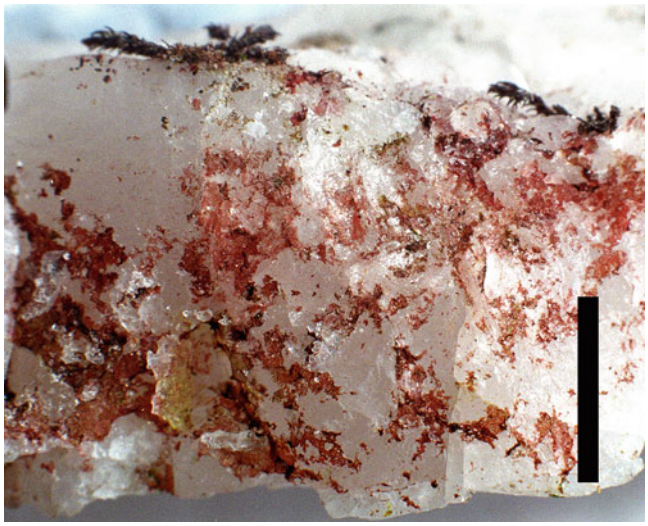

Fig. 10.3 Chasmoendolithic *Gloeocapsa sanguinea* within a quartz vein from Mount Snowdon, Wales. Surface at top of picture with the bryophyte *Andraea*. Bar = 5 mm

Fig. 10.4 Lava boulder at Coniston, Cumbria showing lichens colonizing the ridge and dark cyanobacterial communities covering the sides. Boulder 1 m high

variable periods of time for full metabolic activity. Some subaerial communities are moist for much of the time, such as those in cloud forest or the spray zone of a waterfall. Surface and near-surface cyanobacteria in deserts may be moistened sufficiently by condensation at night for metabolic activity to occur, but only a short period at early dawn when then they retain sufficient water to permit photosynthesis. In spite of Petersen's (1915) original definition of 'aerial' as getting most of the water from the atmosphere, there are few quantitative data about this (Chap. 12) and it seems likely that most water is obtained from liquid form, even if only transitory periods of condensation or snow (Friedmann et al. 1980).

In the Taylor Valley, Antarctica, Büdel et al. (2008) found that the occasional dew and rime formed on granites activated the chasmoendolithic cyanobacterium community. Condensation can be just as important in temperate climates, such as for the chasmoendolithic *Gloeocapsa sanguinea* in Fig. 10.3. When rock in Cumbria, UK, is cooled by night-time radiative loss, it can get soaked in subsequent moisture-laden winds (A.P., unpublished). Small-scale changes in water relations due to such causes can lead to marked changes in the phototroph community. For instance, boulders at Coniston, Cumbria, are dominated by lichens on the xeric summit of the rock as opposed to being dominated by cyanobacteria on the more mesic sides (Fig. 10.4). A similar contrast has been observed on limestone pavements, with lichens predominating at the more xeric sites. This phenomenon becomes less apparent at lower latitudes however.

Light (Sect. 10.2.3), temperature (Sect. 10.2.4) and chemical environment (Sect. 10.3) can interact in various ways to influence how the organisms respond to a particular water regime. The considerable range of species that occurs in subaerial environments is therefore to be expected. It should eventually be possible to relate the several hundred (or probably more) cyanobacterial species listed as subaerial in the various floras to particular microhabitats, which largely reflect different combinations of these four factors. Floristic lists would then provide a good indication of the environment at a particular site.

The great majority of subaerial cyanobacteria possess a thick sheath or other form of extracellular matrix that helps to protect cells from damage by desiccation (Potts 1994). The matrix probably also helps to maintain the 3-dimensional structure of colonies and communities during drying cycles, though there is as yet no explanation for the underlying controls that would permit this (Chap. 18). Lewin (2006) observed that *Gloeocapsa* cells clumped more in drier than moister habitats, perhaps as a water conservation measure. Lewin also suggested that in cases where wetting events are of short duration, cyanobacteria may be incapable of completing a cell division cycle and may need to arrest for an indefinite period. This appears to be the situation for *Nostoc flagelliforme* in semi-desert environments (Shaw et al. 2003; Chap. 12). More information is needed on the features of cell division that help adapt such species to this kind of stress.

Sheaths or less clearly delimited forms of EPS are produced by almost all subaerial cyanobacteria and their importance near the surface of dry region soils is described in more

detail in Chap. 12. However, there are many other examples. For instance, in a greenhouse study, inoculation of an Argentine saline-sodic soil with *Nostoc muscorum* led to a slimy film 3–5 mm thick covering all the surface of the soil (de Caire et al. 1997). Similar results were found when *Nostoc* was inoculated onto the surface of a ferruginous tropical soil from Western Cape, South Africa (Issa et al. 2007). In spite of the importance of EPS in minimizing cell damage when exposed to drying, more diffluent EPS (mucilage) is often most conspicuous under moist conditions, as in the spray zones of waterfalls and the abandoned mines described in Sect. 10.2.3. It was suggested (Fritsch 1907b) that the contrast between the frequency of subaerial colonial cyanobacteria with diffluent sheaths in the cooler uplands of Sri Lanka (Ceylon) with their rarity in the lowlands may be due to the frequent desiccation occurring in the latter. However, the visual prominence of the extracellular matrix does not reflect the amount of material produced per cell and hence the amount of energy required.

Various studies have compared ultrastructural and physiological features of dry cells and ones after various periods of rewetting. In the study of terrestrial cyanobacterial communities in Gimhae, Korea, by Tripathi et al. (2007), several inclusion bodies were more frequent in dry than moistened cells and, in some cases, mainly filaments, there was a distinct discontinuity layer between the sheath and cell membrane. Following a period in a desiccator for 6 months, rewetted *Gloeocapsa* from an intermittently moist position on a limestone cliff at Malham Cove, England, showed detectable CO_2 fixation soon after rewetting, whereas a *Schizothrix* community from of permanently wet position was much slower to respond (Pentecost 1992).

10.2.3 Light

Field observations have shown that subaerial cyanobacteria are capable of growth under extremes of solar radiation. They are abundant on rocks of the moist tropics where photosynthetically available radiation (PAR) levels reach values of 500 $W\,m^{-2}$ (c. 2,000 $\mu mol\ m^{-2}\ s^{-1}$), yet similar forms may also be found at cave thresholds where levels may fall well below 0.1 $W\,m^{-2}$ (0.4 $\mu mol\ m^{-2}\ s^{-1}$) spanning at least three orders of magnitude (Asencio and Aboal 2000; Pentecost and Zhang 2004). Gorbushina (2007) commented on the radiation stress encountered at intervals during the evolutionary history of subaerial organisms, such as sun-spot activities and the extremes encountered during Milankovic cycles of 18,000–100,000 years. In the case of very low PAR levels, nothing is known about the extent to which some of the strains are growing photoheterotrophically (mixotrophically) or perhaps almost entirely heterotrophically.

The most obvious visual difference in the communities in response to light regime is the level of sheath pigments, which provide strong protection from UV radiation and a substantial reduction of the visible part of the spectrum. This topic is considered fully in Chap. 19, but a few details are included here. Sheath colours were reported routinely in some of the early detailed studies on rock cyanobacteria, such as by Ercegović (1925) and Jaag (1945). The most widespread sheath pigment on cells exposed to high irradiance is the brown scytonemin (Proteau et al. 1993), but in some Chroococcales the pigment is gloeocapsin, which is pH-sensitive, being red at low pH and blue at high pH. In general the most strongly illuminated subaerial sites have the most heavily pigmented sheaths, but there are some deeply shaded sites where the sheaths of at least some cyanobacteria are deeply pigmented. A study of *Scytonema* populations in northern England led to the suggestion (Pentecost 1993) that this is a response to slow growth rates, exposing individual cells to UV damage over a long period. It has now become clear that the ability of faster-growing cells to repair damage by mechanisms not involving special pigments is important (Lewin 2006; Chap. 19). The relationship between high irradiation and pigment formation is even less clear-cut in aquatic species, especially planktonic or periphytic ones. However, subaerial environments that are deeply shaded can sometimes support cyanobacterial communities with heavily pigmented sheaths and some rock-inhabiting cyanobacteria synthesize a small content of scytonemin constitutively (Chap. 19). In addition, one of the *Calothrix* strains isolated from UK streams and tested for the their ability to grow heterotrophically in the dark continued to produce scytonemin under these conditions (B.A.W., unpublished data).

The possible taxonomic value of pigmentation has been the subject of considerable debate. Ercegović (1925, 1932) and Jaag (1945) maintained that it was environmentally produced and of little or no taxonomic value. However, although they recognized the importance of light intensity, Anagnostidis and Komárek (1998) and Komárek and Anagnostidis (2005) treat pigment as an important taxonomic character and in some cases the main phenotypic one separating species. Populations of cyanobacteria scraped from a rock surface exposed to high intensity sometimes show a considerable colour range and it may be difficult to decide whether this is environmental or indicates genetic diversity. For instance, the sheaths of a *Gloeocapsa alpina* population may range from blue to dark violet, which might indicate nothing more than pH differences in the microenvironment, but it would require isolation and culture of individual cells to prove this. The evidence so far suggests that species forming scytonemin, such as *G. kuetzingiana* and *Scytonema myochrous*, lack gloeocapsin, while species with gloeocapsin such as *Gloeocapsa sanguinea*, lack scytonemin, although they are often intimately associated on rock surfaces. However, some taxa, such as *G. shuttleworthiana*, have orange-brown sheaths, so perhaps these produce both pigments. Other extracellular pigments

Fig. 10.5 *Schizothrix coriacea* growing in the intermittently moistened zone at the edge of the calcareous River Muga, N-E. Spain. The pink colouration of the sheaths intermingled with calcareous deposit becomes more obvious where moistened, but the moist part of the community shown on bottom right also includes *Rivularia* with brown scytonemin in its sheaths

are included in taxonomic accounts, but apparently remain uncharacterized. For example, subaerial populations of *Schizothrix* often possess a reddish or pink pigmentation when seen in the field (Fig. 10.5), though it is not always easy to see with the light microscope. It is chemically distinct from gloeocapsin (A.P., unpublished data).

The limits for cyanobacterial UV tolerance were tested by Olsson-Francis et al. (2010). Rocks from a limestone cliff in Devon, UK, were launched into low Earth orbit at about 300 km for 10 days to provide exposure to extreme radiation and desiccation. These rocks had a diverse epilithic and endolithic cyanobacterial community composed of Pleurocapsales, Oscillatoriales and Chroococcales. Following exposure, only a single cyanobacterial strain was isolated. The same organism was isolated from the limestone cliff after exposing the rock-dwelling community to desiccation and vacuum (0.7×10^{-3} kPA) in the laboratory. The morphology of the organism indicated Chroococcales and it formed large clumps surrounded by a sheath.

Sheath pigmentation reduces the PAR available for photosynthesis, and the occurrence of pigmented forms in well-illuminated sites and the fact that the cyanobacteria often predominate in low illumination regimes (Chap. 11) suggests that the group as a whole can adapt to moderate or low light intensities. In the cave in S-E. Spain with extremely low light intensities (see above), Martınez and Asencio (2009) found only cyanobacteria – no green algae or diatoms. Although most other authors have reported other organisms in caves, cyanobacteria are the ones found at the lowest light intensities, as, for instance, reported by Poulickova and Hasler (2007) for some caves in the Czech Republic. The two species furthest into a limestone cave in Yorkshire, UK, were *Gloeocapsa punctata* and *Schizothrix perforans*, which occurred 34 m from the entrance, where the relative PAR was 0.004% (Pentecost and Zhang 2004).

There are a number of measurements of light intensity where cave cyanobacterial populations occur, though presumably these were often made at times when the value was near its maximum. Examples include: well-developed colonies of *Aphanocapsa grevillei* in a Yorkshire cave at 1–2 μmol m^{-2} s^{-1} (A. P., unpublished data); *Aphanocapsa castagnei* in a Czech cave at 0.06 μmol m^{-2} s^{-1} (Mulec et al. 2008); *Hapalosiphon intricatus* in a Florida cave at 1–3.5 foot candles (c. 0.2–0.7 μmol m^{-2} s^{-1}: Davis and Rands 1982); *Gloeocapsa* sp. in a cave at 1 lux (ca 0.02 μmol m^{-2} s^{-1}: Cox et al. 1989). Quinçay cave, near Poitiers, France, is colonized by *Geitleria calcarea* where irradiance has been measured on both a daily and seasonal basis (Leclerc et al. 1983). Here, the PAR, measured close to the *Geitleria*, peaked near midday in early spring at 6 lux (c. 0.1 μmol m^{-2} s^{-1}), but averaged about 0.04 μmol m^{-2} s^{-1} annually. Much lower irradiances were reported by Martınez and Asencio (2009). In a small north-facing Spanish cave, cyanobacteria were recorded from surfaces where the irradiance ranged from 0.0008 to 0.06 μmol m^{-2} s^{-1}. These extremely low values are well below the lowest limits reported for oxygenic photosynthetic growth (c. 0.01 μmol m^{-2} s^{-1}; Littler et al. 1985; Raven and Cockell 2006). Five of the 22 cyanobacteria recorded were collected at levels below this limit, but it is unclear if these species were actually growing, or simply deposited there from more illuminated areas. It is evident that there are practical difficulties in obtaining meaningful measurements of PAR in the cave environment. Estimates of the annual integrated PAR would be more useful and only this approach will provide meaningful comparisons between sites and species. The cells of cave-threshold cyanobacteria contain densely packed thylakoids (Cox et al. 1981; Couté and Bury 1988) and increased pigment levels (Mulec et al. 2008), clear adaptations to low light levels. Unlike the low light conditions found in deep water, spectral light quality in cave thresholds is practically invariant with illumination and chromatic adaptation has not been observed. However, this is not the case with the communities developing around lamps and which pose problems for cave managers (Chap. 11). In an English cave illuminated by fluorescent lamps, strata of *Phormidium valderianum* directly exposed to the lamps were pigmented red with phycoerythrin, while strata exposed to radiation reflected from the cave walls and enriched in longer wavelengths were pigmented blue-green and appeared depleted in this pigment (Pentecost 2010).

The genera most frequently found in heavily shaded parts of long abandoned mines in Cumbria and Northumberland, UK, are usually colonial uncellular genera producing abundant mucilage, *Aphanothece*, *Gloeothece*, *Aphanocapsa* and

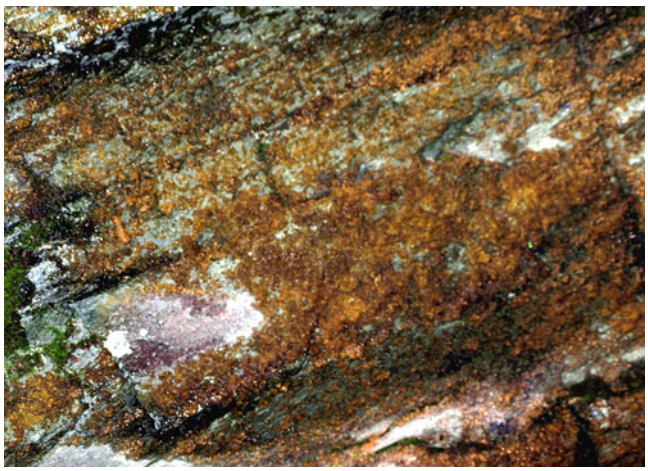

Fig. 10.6 *Aphanothece*-dominated community on wall near the entrance of Rydal Cave, Cumbria, England

Chroococcus. The abundance of mucilage in a moist or wet environment probably permits colonies to remain wet and metabolically active for long periods to take advantage of the low light regime. *Aphanothece* and *Gloeothece* have ellipsoid cells and those of *Aphanothece* (Fig. 10.6), but not *Gloeothece confluens*, at Rydal Cave, Cumbria, were orientated such that their long axes were perpendicular to the light source, which could improve light capture under low illumination (A. P., unpublished).

10.2.4 Temperature

Subaerial cyanobacterial populations, especially those on rock surfaces, often encounter a much wider temperature range than most populations of aquatic cyanobacteria. However, they do not normally experience the upper part of their temperature range when they are in their fully hydrated condition. A moist rock surface, exposed to the sun, evaporates its free water and in the process evaporative cooling takes place. Thus, while rocks remain moist, their surfaces are unlikely to exceed 50°C. In other circumstances they can reach at least 60°C (Gorbushina 2007) and probably more in desert regions (Chap. 12). Cells must be able to adapt to temperature changes rapidly, since a transition from full hydration to near-desiccation can take place in a few hours or even a few minutes, well within a single cell division cycle and with no time to develop thick-walled resting stages.

When a rock begins to dry as a result of insolation, the temperature rises, but the rate of increase depends upon several factors. Among the most important, assuming a constant radiation flux, are the light absorption properties of the surface, its heat capacity and conductivity (Table 10.2). The table shows that the heat capacities and thermal conductivities of rock surfaces do not differ greatly from one another,

Table 10.2 Some heat capacity, thermal conductivity and reflectivity (albedo) values for common subaerial surfaces (Anon 2005)

Material	Specific heat J kg^{-1} $°C^{-1}$	Thermal conductivity W m^{-1} $°K^{-1}$	Albedo %
Concrete	1,000	1.7	55
Granite	790	2.1	35
Limestone	910	1.3	40
Sandstone	830	1.7	–
Slate	–	–	7
Wood	1,700	0.13	–

while wood has a much higher heat capacity and lower conductivity and will retain its heat for longer. The rock albedos, however, show striking contrasts, with limestone and concrete reflecting about half of the incident solar radiation, while dark materials like slate absorb most of it. Some rocks such as basalt and peridotite are naturally dark owing to the minerals they contain, while others become so with inorganic patinas (e.g. Mn oxides). However, many paler rocks also become darker due to the growth of organisms, as is sometimes very obvious due to differences between quarried and undisturbed surfaces. Many dark-coloured crusts on rocks are caused by cyanobacteria and lichens, with the latter frequently containing cyanobacterial symbionts (Chap. 23).

If the surface temperature is lower than the overlying air temperature, solar heating warms darker surfaces more rapidly than paler ones (Hall et al. 2005). If the surface is moist, it should therefore dry out more quickly. However, once the surface temperature exceeds that of the air, the maximum temperature attained is independent of the albedo owing to heat convection in the overlying air. In their outdoor laboratory in Canada, Hall et al. measured maximum surface temperatures of experimental tiles of approximately 80°C irrespective of their albedos. Assuming the phenomenon can be extended to subaerial communities, the main effect of a low albedo (e.g. dark cyanobacterial mat) is to increase the rate of evaporation, leading to a more xeric environment. It would be interesting to establish whether this has any influence on the selective advantage of a particular type of UV protective pigment in a particular microhabitat.

There are a number of direct measurements of temperature from subaerial surfaces with cyanobacteria, though, surprizingly, fewer for high than low temperatures and most of the former for near-desert conditions. Chasmoendolithic cyanobacteria at the threshold of the cave in S-E. Spain studied by Asencio and Aboal (2000) were exposed at different times of year to 1.6–39.1°C at a position where the relative humidity ranged from 2.4% to 77.6%. The temperature range is quite similar to the annual range in air temperature, reflecting the sheltered nature of the site. Bell et al. (1988) reported a mean air temperature of 11.0°C on the Colorado Plateau, USA, where sandstones were colonized by endolithic cyanophytes, but the actual range in surface temperature was probably

substantial. In Iceland, Herrera et al. (2009) recorded temperatures up to 40°C for a cryptoendolithic community inhabiting volcanic glass. Subaerial cyanobacteria also inhabit some of the coldest regions on earth and Wynn-Williams (2000) includes many of the records up to that time. The sandstones of Ellesmere Island, Canada, provide one of the lowest records, with endolithic cyanobacteria present where the mean air temperature is −19°C (Omelon et al. 2006). Such extremes indicate that ice-nucleation inhibitors must be present within the cytoplasm.

Although aspect has sometimes been included in notes about sites (e.g. Jaag 1945), its influence on subaerial cyanobacteria has seldom been considered in any detail. Nevertheless aspect has an influence on light, temperature and perhaps especially water regime due to the effect of wind. Broady (1981) found that cyanobacteria developed preferentially on western to south-western slopes in Princess Elizabeth Land and MacRobertson Land, Antarctica, and that this was related to wind direction rather than light. He also reported that these epiliths only occurred where snow drifts provided films of liquid water during summer. Abrasion by snow and ice occurs on the windward slopes of rocks, whereas the accumulation of snow on the lee sides provides moisture for phototrophs when it melts in summer. It would be interesting to establish the extent to which it is possible to generalize about the effects of aspect on subaerial surfaces and hence phototroph growth. It is known that east-facing rock surfaces tend to be more mesic than west-facing ones, which become exposed to the sun later. Water condensed on rock surfaces at night is evaporated later on west-facing rocks, because the highest surface temperatures are reached in the afternoon, with a period of more rapid evaporation.

10.3 Chemical Environment

10.3.1 pH and Inorganic Carbon

The hydrogen ion activity of rock water films depends upon both the rock chemistry and the origin of the water film. Rainwater pH is usually in the range 4–5 (Stumm and Morgan 1996), resulting from the dissolved atmospheric CO_2 that becomes partially hydrolysed to HCO_3^-, plus traces of acid-forming gases such as NO_x and SO_2. Upon contacting a rock surface, reactions occur that can result in the release of (more) HCO_3^- ions. Carbonate rocks, particularly limestones, are well known for their rapid reaction with precipitation (Brook et al. 1983), with the release of Ca^{2+} and HCO_3^-. Soil waters are generally even more reactive, since they contain higher concentrations of dissolved CO_2 via soil respiration that act as an acid, resulting in the formation of water containing up to 5 mM L^{-1} HCO_3^-.

Due to the wide range of rock-forming minerals, water-rock reactions can only be considered in broad terms. Only quartz, the common feldspars and calcite are considered here, since they are major constituents of many rock types. Quartz, consisting of silicon dioxide, is slightly soluble in water forming monomeric 'silicic acid' in the micromolar range, providing the pH is <8 (Stumm and Morgan 1996). Water pH is hardly affected by the dissolution and cyanobacteria are not known to metabolise silica, so quartz is assumed to be an inert substratum for these organisms, although soluble silica chelates are produced by some soil microbes, enhancing quartz dissolution (Bennett and Siegel 1987). Feldspars, consisting of a range of what are essentially substituted metasilicates (in effect SiO_2 where some of the Si is replaced by Al with additional Na, K or Ca) are more reactive towards water and particularly the dissolved CO_2, resulting in the release of HCO_3^- and OH^- accompanied by a rise in pH. As a result of these and other processes, rock surface pH may be expected to vary over a wide range, from about 2 on rocks containing reactive sulphides such as pyrite to 8 or more on limestones. Field observations indicate that subaerial cyanobacteria are more prevalent on circumneutral to alkaline substrata (Pentecost and Whitton 2000), but there are many records of them colonizing sites at much lower pH values. For instance, *Stigonema* can be common on rock seepages in the pH range 4.4–8.1 (Woodhead and Tweed 1954). The lower pH limits for cyanobacteria in aquatic environments are discussed further in Chap. 1.

Carbonate rocks such as limestones provide a ready supply of dissolved CO_2 through interaction with water and its dissolved acids. Although limestones are considered to be the main source of calcium-and carbonate-containing minerals, other rocks often contain significant amounts such as basalts, dolerites and granites. Carbon acquisition by cyanobacteria is discussed in Chap. 17, but one aspect needs comment here. Rubisco, the enzyme in cyanobacterial carboxysomes responsible for carboxylating CO_2 in photosynthesis has only a moderate affinity for CO_2, with typical k_m values >150 μM (Price et al. 2002). As a consequence, a number of carbon-concentrating mechanisms (CCMs) have been proposed to increase the CO_2 levels in carboxysomes and thus make photosynthesis more efficient (Badger et al. 2002; Price and Badger 2002). Among the small number of cyanobacteria that have been studied, at least four transporters have been found: low affinity CO_2, high affinity CO_2, low affinity HCO_3^- and high affinity HCO_3^-. High affinity uptake mechanisms are required where the external carbon species concentration is less than 20–50 ppm (Price et al. 2002). In a typical rainwater in contact with limestone in the UK, the CO_2 is around 5 mg L^{-1} and HCO_3 around 50 mg L^{-1} (A.P., unpublished), showing that high affinity transporters would be required for CO_2 and possibly HCO_3^-. On carbonate-poor rock such as leached sandstone, HCO_3^- concentrations would be considerably less.

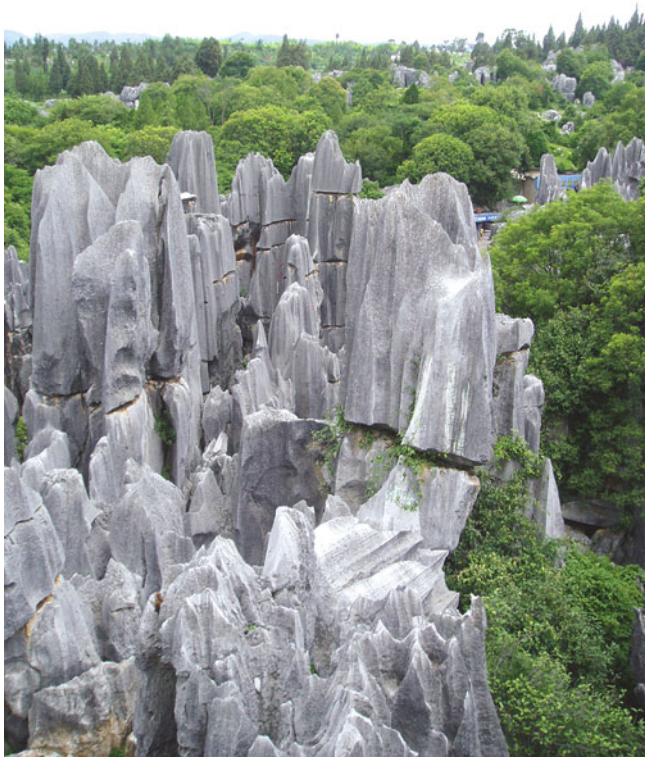

Fig. 10.7 Epilithic *Gloeocapsa alpina* covering surface on limestone pinnacles of the Yunnan Stone Forest, SW. China

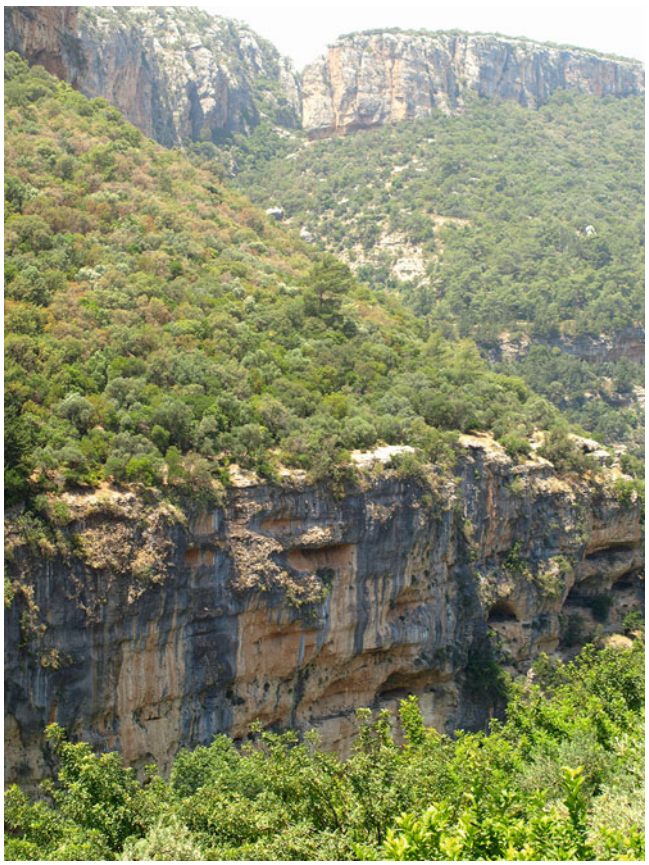

Fig. 10.8 Vertical streaks (tintenstriche) dominated by *Gloeocapsa alpina* on the limestone cliffs of Limonyu Canyon, SE. Turkey

Molecular studies have identified two types of carboxysome in cyanobacteria, associated with different combinations of transporters, leading to the recognition of α- and β-cyanobacteria (Badger et al. 2002). The α-cyanobacteria appear to be adapted to more stable environments such as the open ocean, while β-cyanobacteria possess all four transporters and appear better able to respond to sudden changes in CO_2 or HCO_3^- in the external environment. The strains so far studied appear to originate from aquatic environments and it will be of interest to see where the subaerial cyanobacteria stand in this respect.

Red *Gloeocapsa* (e.g. *G. sanguinea*, *G. magma*) containing gloeocapsin are colonists of mesic acidic rocks (Fig. 10.1), while the 'blue' species (*G. alpina*, *G. compacta*) are common on limestones (Figs. 10.7 and 10.8), but microscopy often reveals both forms growing together, with one predominating. It is possible that the localised colour differences are due to microenvironmental variations in pH rather than different species being present. This could result from local differences in photosynthesis or respiration rates, or differences in mineral composition. If only a single species is involved, growing equally well on acidic and calcareous rock, it must have at least two transporters, one for CO_2 (on acid rock) and one for HCO_3^- (for limestone surfaces). If this is the case, gloeocapsin-pigmented *Gloeocapsa* is a remarkably successful subaerial organism.

10.3.2 Major Metallic Elements

Significant quantities of Ca are released from limestones in contact with rain- or soil water. Calcium concentrations in the range 1–2 mML^{-1} are often observed (Stumm and Morgan 1996), whereas Mg is typically an order of magnitude less, though the ratio between the two elements can differ greatly according to type of limestone. The external Ca^{2+} concentration in calcareous environments may be 1,000 times higher than the intracellular one. Passive influx of the ion is probably negligible and it seems likely that any influence of high Ca concentration on rock colonization is mainly indirect due to pH and hence availability of other elements, ions or compounds. In an experimental study on *Nostoc* an increase in external Ca from 10 to 100 μM doubled the heterocyst frequency (Onek and Smith 1992), but even the upper concentration is probably below the lower limit at the site where the organism had grown in nature. The subaerial cyanobacterial

flora of dolomite (a calcium magnesium carbonate) in the UK (authors, unpublished) and the Alps (Jaag 1945) does not appear to show major differences to that of limestone.

The geochemical paradox that a few species of cyanobacteria can bore into limestone surfaces, whereas photoautotrophs in general may be expected to favour limestone deposition was investigated for a marine species by Garcia-Pichel et al. (2010). Strain BC008, which was used for this, had been obtained during an earlier study (Chacón et al. 2006) of microboring cyanobacterial communities in marine carbonates in Puerto Rico. No taxonomic name was given by Garcia-Pichel et al., but their figure shows an organism with true branching and cells of variable width. The organism was able to take up Ca^{++} at the excavation front, decreasing the local extracellular ion activity product of calcium carbonate enough to promote spontaneous dissolution. Intracellular Ca^{++} was then transported along the branched trichome and excreted at the distal end at the borehole opening into the external medium. Inhibition assays and gene expression analysis indicated that uptake and transport were driven by P-type ATPases. Transport depended on illumination, with the rate increasing at higher light intensity. Although the authors did not suggest it, it seems likely that the Stigonemales genera recognized as *Brachytrichia* and *Kyrtuthrix* behave in a similar way.

Na and K can be released in small amounts through rock weathering, but in many regions rainwater carrying dissolved aerosols originating from ocean spray is likely to be more important. The influence on subaerial cyanobacteria in general is probably minor, but on impervious rock surfaces where small rain puddles form, locally extreme salinities will exist as the last trace of water evaporates. In addition, continued wetting and drying could gradually increase the salt content in small depressions. Presumably this could stress the associated cyanobacteria and select for resistant strains; it may also account for the occasional record in tiny pools at inland sites of *Johannesbaptistia*, a genus normally associated with coastal regions (Whitton 2011). Such environments become more frequent near the sea and merge with the subaerial communities of the marine supralittoral zone. It may also account in part for the pronounced zonation of cyanobacteria and cyanolichens around limestone solution hollows.

Examination of coarsely crystalline silicate rocks such as granite should provide clues to their influence upon cyanobacterial colonization. A comparison of the terrestrial lithophilic algae in two granite canyons in Ukraine found that 22% species in the steppe zone were cyanobacteria, but only one species (*Phormidium autumnale*) in the forest zone, as opposed to 67 eukaryotic algae in this zone (Mikhailyuk 2008). Presumably the organisms were influenced by the granite more directly in the steppe zone. Quartz, feldspar and a range of ferromagnesian minerals are often easy to distinguish, so selective colonization should be apparent. The actinomycete *Streptomyces* can attack the ferromagnesian mineral hornblende with the aid of a catecholate siderophore (Liermann et al. 2000) and some cyanobacteria also possess siderophores containing a catecholate moiety (Gademann and Portmann 2008), so perhaps they can also metabolize iron from these minerals. However, observations on the Shap granite of Cumbria, England, and Lewisian Gneiss of Scotland have failed to demonstrate differences in colonization by red *Gloeocapsa*: quartz, orthoclase feldspar and the ferromagnesian mineral hornblende appear to make equally acceptable substrates (A.P., unpublished). A different story could emerge with more porous finer-grained rocks, where the area of mineral/water contact is much higher.

10.3.3 Nitrogen and Phosphorus

The water regime has a major impact on the nutrient regime at a site, but quantitative data are sparse. Overall, the availability of P is the most important factor for subaerial cyanobacteria, because of the ability of some taxa to fix N_2. Soil drainage and seepage zones are probably responsible for most of the nutrient supply where prominent cyanobacterial streaks (tintenstriche) occur on cliffs (Fig. 10.8). It seems likely that most nutrients in seepage originate from surface drainage, but some may also be derived from the rock, especially if it is limestone. A study (Davis and Rands 1982) of a limestone cave in Florida with a resident bat population provided a comparison of the composition of the limestone underlying *Hapalosiphon intricatus* growths with solutions in equilibrium with limestone samples. The P content of the underlying limestone was 0.85 μg g^{-1} P, plus 1–6 μg g^{-1} NO_3-N and 3–10 μg g^{-1} NH_4-N). The value for the equilibrium solution for P was 1.6–6.5 μg L^{-1} P. The authors suggested that the combined N could have been derived partly from seepage, with NH_4-N also coming from the bats and N_2 fixation by *Hapalosiphon*.

Nutrients may also reach near-horizontal surfaces by upwelling through porous limestone. Although the composition of the water is probably quite similar to that of any seepage water in the vicinity, there is an increased possibility of ions such as phosphate being precipitated because of the close contact with the limestone. In this situation it is especially likely that most of the phosphate will be organic because of inorganic phosphate being precipitated as calcium phosphate. Several eukaryotic algae such as *Oocardium* seem to favour this microhabitat, but it is unclear how widely this applies to cyanobacteria, though it may influence colony structure of species such as *Scytonema myochrous* growing on loose calcareous deposits. Possibly the availability of organic phosphate is a factor explaining why *Nostoc* colonies (Fig. 10.10) and the *Nostoc* cyanolichens *Collema* and *Leptogium* are more common on limestone surfaces in contact with soils than those which are not.

10.3.4 Minor Elements

A study (Ferris and Lowson 1997) on vertical dolomitic limestone cliffs in Ontario showed that rock in the vicinity of an endolith community was enriched in some elements (e.g. P, Ba, Zn, Pb) and depleted in others (e.g. Mg, Fe, Cu). The authors interpreted this as evidence that the endolithic microorganisms were playing an active role in biochemical cycling of nutrient and trace elements at the site. However, caution is needed in interpreting the data from this and other analyses.

10.4 Habitats and Communities

10.4.1 Cliffs and Slopes

The descriptions of subaerial cyanobacterial communities in the literature mostly rely on the use of classical names, so their diversity depends to some extent on the experience of the researcher. Jaag (1945) is still an important source of information for all types of rock, though based especially on his studies in Switzerland. Among more recent detailed accounts of epilithic cyanobacteria is that by Hoffmann (1986) for Luxembourg. Cyanobacteria typically show more taxa and greater biomass on limestone than other surfaces, as found, for instance, by Sokoll (in Strzelczyk 1981) in a comparison of limestone and sandstone.

A few researchers have adapted the Braun-Blanquet terminology developed for higher plants to describe subaerial cyanobacterial associations, the most comprehensive being Golubić (1967b) for the limestone of the Dinaric Alps. The associations recognized reflect the different combinations of two of the major environmental variables discussed above, water and light. Under high irradiance, increasing 'wetness' leads to change from a *Scytonemo gloeocapsetum* to a *Tolypothricetum byssoideae* and finally a *Dichothricetum compactaea*. Characteristic species of the first (most xeric) association include *Scytonema myochrous*, *Gloeocapsa compacta*, *G. kuetzingiana* and *G. sanguinea*, while the wettest sites had *Dichothrix compacta*, *Phormidium autumnale* and *Xenococcus kerneri*. Extensive mats of *Scytonema myochrous* were reported from the Pedras Nigras de Pungo Andigo mountains of Angola by Fritsch (1907a), and modern internet pictures suggest these rocks have a conspicuous cyanobacterial cover.

Calcareous sites with lower illumination and intermittent moisture, especially where the wet periods tend to be quite frequent, are often dominated by *Schizothrix* spp. Such communities have been observed by the authors at many sites in the UK and other countries, and similar communities were reported by Fjerdingstad (1957). *Pseudocapsa dubia* was first described by Ercegović (1925) from rocks in Croatia as a new species, but it has since been found to be quite widespread on moist, shaded calcareous surfaces.

Much less is known about the communities of non-calcareous rocks and in any case the composition of the rock is often uncertain in older accounts for particular sites or general floristic lists, such as that by Fritsch and Rich (1924) for subaerial algae in the Tugel Gorge, Natal, S. Africa. Among species recorded by them from cliffs and other rock surfaces were *Stigonema informe*, *S. hormoides* var. *africana*, *S. ocellatum*, *Calothrix parietina*, *Schizothrix muelleri*, *S. epiphytica* and *Gloeocapsa sanguinea*. *Scytonema myochrous*, *Gloeocapsa alpina* and *G. punctata* were noted on moist and dripping cliffs; the last was found in rock crevices, while first two are limestone species not only here, but in most places where they occur. The four genera on the sandstone rocks of eastern Venezuela studied by Golubić (1967a) were, in decreasing order of importance, *Gloeocapsa*, *Schizothrix*, *Stigonema* and *Scytonema*; all had coloured sheaths.

The littoral zone of lakes and rivers provides an environment where aquatic and subaerial species may exist in close proximity. There have been several classifications of the littoral environment (eg. Wetzel 2001) and the uppermost zones, termed the supralittoral and epilittoral is that influenced by wave splash or spray respectively, may be considered part of the subaerial environment. Rocky, oligotrophic lakes in the western part of the British Isles frequently possess a 'black' supralittoral and epilittoral zone up to 2 m above the mean water level (Fig. 10.9), where a variety of dark-pigmented cyanobacteria and lichens occur. Common constituents include *Gloeocapsa kuetzingiana*, *G. sanguinea*, together with species of *Scytonema* and *Stigonema*, the latter often lichenized as *Ephebe lanata*. It is unclear whether distinct zones of these taxa are responding to changes in exposure, as is the case with the littoral lichens.

Colourful vertical streaks of cyanobacteria can be found on steep cliffs worldwide and are associated with temporary water runnels, which may be considered as extreme examples of ephemeral streams. The streaks develop on many lithologies, but hard impervious rocks are especially favoured, as these tend to provide stable cliff faces which permit water to flow, rather than to be absorbed. These tintenstriche (= ink streaks) were first described in detail by Jaag (see his 1945 review for references), who found that the cyanobacterial community differed markedly according to rock lithology. Fjerdingstad (1965) and Luttge (1997) provide updates. However, these communities have not yet been defined climatically (number of wet days, temperature range, irradiance), nor have they been analysed according the water status (period wet enough to metabolize and period influenced by flowing water with any associated nutrients). On drier limestones such as steep cliffs receiving only temporary inundation, the communities consist mainly of coccoid forms and often take a long time to colonize. The significance of water

Fig. 10.9 Dark zone of encrusting cyanobacteria at the edge of oligotrophic Lough Uachtarach, Killarney, Ireland. Cyanobacteria include *Gloeocapsa kuetzingiana*, *G. punctata*, *G. sanguinea* and *Stigonema hormoides*, with occasional *G. compacta*, *Microcoleus chthonoplastes*, *Stigonema minutum* and the cyanolichen *Ephebe lanata*

was examined by Viles (1995), who provides a conceptual model of the efficiency of biological weathering by microorganisms as a function of water stress, with epiliths becoming more important than endoliths as water availability increases.

The weathering of rocks by microorganisms has been recognised for more than a century (Viles 1995) and Meybeck (1987) calculated rates of chemical weathering on different rock types based on the dissolved loads in rivers. Meybeck's estimates indicated that the rate on carbonate rocks was some 12 times that on granite and measurements of limestone weathering suggest that cyanobacteria are in some places quantitatively important in this process. In Yorkshire, UK, fragments of limestone cliff up to 2 g weight were dislodged beneath a thick tintenstriche of *Gloeocapsa* and *Scytonema* spp., equivalent to an overall surface weathering rate of about 3 mm/100 years (Pentecost 1992). In such cases it is probable that the dehydration and rehydration of the sheaths assisted in the loosening of the surface. It has also been argued that a covering of cyanobacteria can actually protect rock surfaces by smoothing out variations in water content and temperature (Darlington 1981), although in the case of the latter this seems unlikely.

10.4.2 Horizontal Surfaces

Horizontal surfaces permit growth of loose colonies of *Nostoc commune* (Fig. 10.10) and a few other taxa, which would be unlikely to persist on vertical surfaces. Some of the species of vertical surfaces can also occur, such as the *Gloeocapsa* cover on limestone scree in many parts of the world, including that in the Yorkshire Dales, UK, where it results in a location acquiring the name Grey Gill. However, chemical and biological weathering often leads flat limestone

Fig. 10.10 Dry *Nostoc commune* colonies at edge of car-park by Lough Derravaragh, Co. Westmeath, Ireland, in August 2009

surfaces to develop shallow hollows or other irregularities, which provides a greater range of microhabits. In addition, limestone surfaces may be expected eventually to develop a higher plant cover in most types of environment. Many studies have reported cyanobacteria to be important in the weathering of terrestrial limestone surfaces, often in association with other organisms, such as the combined effects of cyanobacteria and lichens reported for hillsides in the Negev, Israel (Danin and Garty 1983). Weathering due to cyanobacteria involves a variety of processes, including the growth of endoliths. Sometimes, the latter may be considered opportunistic, making use of cracks and holes already present, such as with the endolithic community dominated by *Gloeocapsa* in the dolomitic limestone studied by Ferris and Lowson. However, boring by cyanobacteria may also play a role (Viles 1987).

The processes and cyanobacterial flora of terrestrial environments on Indian Ocean atolls has been described in a number of accounts. On Aldabra Atoll, Viles (1987) observed a limestone 'biorind' a few millimetres thick, where surface precipitation and dissolution processes were apparent. Much of the surface of Aldabra (Whitton 1971), Astove and Farquhar not covered by higher plants is covered by free-living cyanobacteria, with *Tolypothrix byssoidea* quantitatively the most important on highly eroded surfaces (champignon) and drier parts of flat surfaces (platin). *Nostoc* colonies are abundant on flatter surfaces with a frequent cycle of wetting and drying. In contrast, the surface of St. Pierre, a raised atoll which has been mined extensively for guano, is largely covered by lichens (Whitton and Donaldson 1977). The explanation for this difference is still unclear. Petersen (1928) reported *Phormidium subfuscum* from guano-enriched rocks.

Floristic accounts of subaerial cyanobacteria from predominantly horizontal surfaces include Petersen (1928) for Iceland, Trono (1961) for the Philippines and Branco et al. (2009) for (non-calcareous) stones on the floor of Atlantic rainforest, São Paulo State, Brazil (see also online article related to this book). Branco described over 20 species of Chroococcales and Oscillatoriales together with some environmental variables. *Gloeothece confluens* grows on the sandstone rock in the Weald of Kent and Sussex, UK, but the flora of these is largely dominated by coccoid green algae (Pentecost and Rose 1985).

10.4.3 Endoliths

Cyanobacteria occur as endoliths throughout the world within a range of rock types and they have been classified according to their mode of occurrence within rocks (Fig. 10.3). Golubic et al. (1981) recognised three forms: cryptoendoliths growing within porous rocks such as sandstones containing a network of open spaces through which the cyanobacteria may enter and grow; chasmoendoliths that occupy surface cracks or pits in the rock and euendoliths that actively etch away the rock surface by chemical action. In addition, the endolithic habit can originate by burial of the organism in accumulating sediment such as sand or calcite (e.g. during travertine formation) where the term 'interrolith' may be applied.

Although limestone appears to be particularly favoured with several species occurring as euendoliths and chasmoendoliths (Ercegović 1925; Asencio and Aboal 2000), some sandstones and other rocks also support cryptoendolithic and chasmoendolithic communities (Chap. 12). Those of northern Arizona, for example, have a community of *Chroococcidiopsis* and *Chlorosarcinopsis* in regions where the vascular plants consist of cold desert shrub, but change to coccoid green algae where the vascular vegetation is cold temperature forest (Bell et al. 1988). On the sandstones of Ellesmere Island, Canada, where the mean air temperature was −19°C, Omelon et al. (2006) also recorded cyanobacteria belonging mainly to the Chroococcales (*Aphanothece*, *Chroococcus*, *Gloeocapsa*, *Synechococcus*). These experienced slightly warmer and moister conditions than the rock surface. Although the Antarctic dry valleys (Nienow and Friedmann 1993; Wynn-Williams 2000) are at a similar latitude, the much more extreme continental climate further reduces the flora and metabolic activity. Broady (1981) investigated quartzites along the coast of Princess Elizabeth Land, Antarctica, and found that endoliths were more widespread than epiliths.

Schizothrix perforans is a well known endolith in submerged limestones, but is also found as a chasmoendolith in the subaerial environment of cave thresholds (Asencio and Aboal 2000). In addition, *Leptolyngbya gracillima* (*Plectonema gracillimum*) formed a chasmoendolithic patina with *Gloeocapsa kuetzingiana* and *Pseudocapsa dubia* on calcareous rock surfaces. They concluded that while *Schizothrix perforans* was a strict endolith, the majority of endolithic cyanobacteria grew as epiliths where conditions were favourable. Al-Thukair and Golubic (1991) showed that the small cells (baeocytes) of *Hyella immanis* were motile and phototaxic. Their small dimensions no doubt assist movement within the confined spaces of the substratum. Many Chroococcales also produce minute cells under some conditions, but Ercegović (1925, 1932) and Asencio and Aboal (2000) noted that in several species (e.g. *Gloeocapsa biformis*, *G. kuetzingiana*, *Leptolyngbya gracillima*) cell size is actually greater in the endolithic forms. However, the reverse was apparent for *Pseudocapsa dubia*.

Asencio and Aboal (2000) found that chasmoendolithic cyanobacteria (mostly Chroococcales) growing in caves and hollows in Spain were exposed to irradiance ranging from 0.3 to 1,400 μmol m^{-2} s^{-1} (see also Sect. 10.2.3). This is a wide range and most endoliths would experience levels much lower than 1,400 μmol m^{-2} s^{-1} owing to absorption/reflection of radiation by the substratum. The light absorption of microcrystalline limestone is high, and photosynthetic activity by cyanobacteria has been shown to be low at a depth of 2 mm below the surface (Pentecost 1978). Ercegović (1925) and Asencio and Aboal (2000) note that endoliths often form a layer up to 3 mm deep within the substratum and it could be much less in dark rocks such as basalt containing an abundance of ferromagnesian minerals. For example, Herrera et al. (2009) found that photosynthetic activity occurred to a depth of 0.25 mm in a volcanic glass from Iceland. Rocks formed of large colourless crystals such as quartz and ice may possess endoliths to greater depths. For example, Büdel et al. (2008, 2009) observed coccoid cyanobacteria growing at a mean depth of 4.49 mm below the surface of granite and 4 mm in marble in Antarctica. The large UV-lucent quartz veins on (Mount) Snowdon, Wales, possess chasmoendolithic

Table 10.3 Ten most frequently reported genera of cyanobacteria (left two columns) and species (right three columns) in recent literature from the cave environment

Genus	Times reported	Species	Times reported
Oscillatoria	41	*Gloeocapsa punctata*	6
Phormidium	40	*Phormidium foveolarum*	6
Gloeocapsa	38	*Aphanocapsa grevillei*	5
Chroococcus	21	*Chlorogloea microcystoides*	5
Lyngbya	20	*Chroococcus minutus*	5
Nostoc	17	*Chroococcus turgidus*	5
Aphanocapsa	11	*Gloeocapsa magma*	4
Plectonema	11	*Gloeocapsa alpina*	3
Gloeothece	10	*Gloeocapsa granosa*	3
Synechocystis	8	*Gloeothece rupestris*	3

Majority of data refer to European cave thresholds

Gloeocapsa sanguinea to at least 10 mm within the rock (Fig. 10.3).

Some tree barks (e.g. *Betula* spp.) approach transparency and algae often occur below the surface and are usually associated with lichens, but no cyanobacteria appear to have been reported from this endoxylic habitat. Cyanobacterium endophytes are nevertheless well known from several terrestrial bryophytes such as *Anthoceros*, the angiosperm *Gunnera* and the roots of some cycads (Meeks et al. 2001; Bergman and Osborne 2002).

10.4.4 Caves

Light quality and duration are the most obvious factors influencing cyanobacterial diversity in caves and many studies have dealt with one or the other of two well-defined environments, the cave threshold where solar radiation penetrates to varying degrees, and the 'lampenflora' deeper within caves under artificial lighting. In show caves cyanobacterial growth can reach nuisance levels, discolouring calcite formations, and occasionally damaging prehistoric artwork (Lefévre 1974; Chapman 1993), though the problem is increasingly being brought under control in the most important tourist sites (Chap. 11).

At the thresholds of limestone caves, cyanobacteria must compete for light with other algae, bryophytes and ferns, but in the deepest recesses of caves they are usually the sole phototrophs. Water relations in caves are as important to growth and colonization as they are in the open air and some caves are surprisingly dry, supporting virtually no phototrophs. However, most caves, at least in Europe, are damp and with their threshold walls covered with a green algal felt. In excess of 200 cyanobacterial taxa have been reported from caves (Claus 1962; Tian and He 1996; Vinogradova et al. 1998), though there is likely to be much synonymy among the names. Table 10.3 lists the ten most frequently reported genera and taxa based upon a recent literature search. The majority of records are probably from thresholds in temperate regions, but not enough information was available to distinguish threshold floras from lamp floras. The preponderance of Chroococcales is similar to the situation found in other subaerial locations in temperate regions. However, sampling details are rarely mentioned and some collections may have been taken from flowing water. A scarcity of heterocystous species is also apparent, only *Nostoc* being frequently recorded. This may result from the high energy demands of N_2 fixation, although the extent to which non-heterocystous N_2 -fixing strains occur in this habitat is not known.

In Croatia the most frequent species reported by Golubić (1967b) were *Aphanocapsa grevillei*, *Chroococcus turgidus* var. *spelaeus* and *Gloeocapsa kuetzingiana*. Similar associations have been noted in China (Tian and He 1996) and the UK (authors, unpublished). A study of species diversity in an ancient cave in Israel showed that the proportion of coccoid to filamentous forms decreased as irradiance got less (Vinogradova et al. 1998). Twenty genera and 42 species were found in the cave and generic and species diversity both decreased with decreasing irradiance. Caves possess some unusual cyanobacteria not apparently reported elsewhere (Claus 1962). Among these are *Baradlaia speluncaecola*, *Fortiella subaiana*, *Palikiella elegans* and *Spelaeopogon lucifugus*. *Baradlaia* is a member of the Stigonematales and unusual in the possession of sessile heterocysts. Apart from isolated records, there is little information about cyanobacteria in non-calcareous caves such as lava tubes and subaerial sea caves.

What appears to be a unique cave community has been reported from the famous Deer Cave in the Mulu National Park, Sarawak (Lundberg and McFarlane 2011), where horizontal stromatolites are formed on the walls near the entrance. Irradiance is low, but nutrient concentrations are high because of an overlying shelf rich with guano formed by bats. The stromatolites are 15–20 cm deep, 4–7 cm thick and of variable width, generally about 50 cm. The structure of the stromatolite depends on a dynamic equilibrium involving accretion on the upper surface and erosion on the underside; the latter results from guano and biological detritus lodging in the lee of the stromatolite lip, causing local acidification and erosion. The stromatolites are composed of alternating layers of more porous and more dense amorphous (P-rich) hydroxyapatite. The most recent surfaces have living filamentous, coccoid and rod-shaped cyanobacteria, though unfortunately no floristic data were reported.

10.4.5 Leaves, Bark and Animals

Terrestrial plants provide a major exploitable surface for algae. With evidence of extensive forest cover extending back at

least to the Carboniferous (300 Ma), vascular plants may have played an important role in the evolution of subaerial algae since this period. An estimate of the relative surface area of bark in English oak woodland, for example, is about four times the plane surface area of the forest floor with the accompanying leaf surface area being 5–10 times the surface area (Pentecost 2010). It is evident that in well-wooded regions, plant surfaces attain major significance.

The surface of bark is more varied in its water-retention properties than that of the leaf owing to increased porosity with age and the lack of a water-repellent cuticle. This, combined with the often cracked and irregular surface of bark provides a more mesic environment than the typically smoother, non-porous leaf surface, which is subject to rapid desiccation by wind and sun. In addition, leaves of trees are usually shed in periods of a few months or years, while the bark is normally a long-lasting surface of the living tree. One might therefore expect bark to more favourable for colonization by cyanobacteria than leaves. The light climate within forest canopies is usually such that the trunks of the trees are the most shaded providing a suitable habitat for the Chroococcales and Oscillatoriales. In the UK, epiphyllous algae (mainly eukaryotic Chlorococcales) are normally only visible on surfaces of old evergreen leaves of conifers or *Ilex* in the understorey of forests, while they occur in abundance on the adjacent bark of both evergreen and deciduous species. Wylie and Schlichting (1974) investigated the corticolous algae of North Carolina and found that the gymnosperms supported green algae almost to the exclusion of cyanobacteria. By culturing bark algae from a range of trees they failed to find any significant associations between tree species and the cyanobacteria present, the most frequently recorded genera being *Anacystis* (*Chroococcus*), *Lyngbya* and *Nostoc*. Foerster (1971) also failed to find any association between tree species and algal taxa in his study of the elfin forest of Pico del Oeste on Puerto Rico, but did note that the cyanobacteria appeared to occupy an intermediate position in the canopy, suggesting avoidance of high irradiance. Nurul Islam (1972) found that some tree genera such as *Ficus* appear to be more closely associated with cyanobacteria than others, possibly owing to favourable bark characteristics.

In the humid forests of the Sri Lanka mountains, Fritsch (1907a) found *Gloeocapsa* (especially *G. sanguinea*), *Gloeothece*, *Aphanocapsa*, *Nostoc* and *Stigonema.* They were all abundant on the trunks of trees, where colonies sometimes hung down in large jelly-like masses, but differences between phorophytes were not mentioned. A study a century later on algae on bark and decaying wood in lowland forests of Singapore (Neustupa and Škaloud 2010) found 12 cyanobacteria, as opposed to 39 green algae and five Heterokontophyta. Seven of the cyanobacterial taxa were only recorded at one of the seven sites. The authors commented on floristic differences between bark and decaying wood, but it is unclear whether this applied to cyanobacteria.

A list of corticolous cyanobacteria is provided in Table 10.4 where it can be seen that all the major orders have common representatives; unfortunately, neither the substratum nor the host species was described in the original account in many cases. There are at least a dozen records of Chroococcales, particularly *Chroococcus*. Most of these genera are also widespread on rock surfaces, but the occurrence of *Merismopedia* is unusual as it is not normally considered a subaerial genus. *Porphyrosiphon notarisii*, a common filamentous epiphyte in Bangladesh (Nurul Islam 1972), is a widespread species in subaerial and freshwater habitats in the tropics and subtropics, though most frequent on non-calcareous surfaces which alternate between being very wet and dry (B.A.W., unpublished). No records of cyanobacteria on gymnosperms appear to have been made. In the cool temperate woodlands of the UK, cyanobacteria occasionally form colonies on the bark of trees with a circumneutral pH, such as *Fraxinus excelsior* and *Acer pseudoplatanus*. Abundant growths are sometimes associated with seepage tracks from tree hollows containing detritus or from sap weeps. Epiphyllous algal floras appear to be dominated by chlorophytes in temperate climates (Fritsch and Rich 1928; Hirose and Akyiama 1967). However, there is a general impression that cyanobacteria are more prevalent in tropical than temperate forests, but more information is needed. In cold regions such as Iceland, Petersen (1928) noted a paucity of algae on trees and attributed it to the effects of extreme exposure to desiccation.

Animals can provide a subaerial surface suitable for algae and the best known examples for cyanobacteria are those forming part of the diverse microbial community of the hairs of sloths (Wujek and Cocuzza 1986; Wujek and Lincoln 1988), although recent interest has focussed on the green alga *Trichophilus* (Suutari et al. 2010). The occurrence of uncellular cyanobacteria in the hairs of polar bears at a Californian zoo (Lewin et al. 1981) got world-wide publicity at the time, but the problem was largely solved by ensuring that nutrient levels were kept low in the water of the enclosure. Human skeletal remains can support growths of *Loriella osteophila* (Borzi 1892).

10.4.6 Bryophytes

The total surface area of mosses is often an order of magnitude higher than that of the substrate colonized by the moss (Pentecost 1998) and so provides a potentially important habitat for cyanobacterial epiphytes. One of the earliest studies was that by Fritsch (1907a), who observed tangled masses of *Tolypothrix* colonizing bryophyte stems and using them as a support in the forests of Sri Lanka (Fig. 10.11). Presumably

Table 10.4 Corticolous cyanobacteria. Taxa associated with bryophytes and other epiphytes, as well as cyanolichens have been excluded

Order/genus	Species (C) = common	Bark/leaf	Hosts (G gymnosperm; A angiosperm)	Location/comments	References
Chroococcales					
Aphanocapsa	*muscicola*	bark	*Phillipia, Acer* (A)	Rwenzori, Uganda; Cumbria, UK.	Pentecost (unpublished)
Chlorogloea	sp.	bark	Palmae (A)	Spain	Komárek and Anagnostidis (2005)
Chroococcus	*dispersus*, *minor*, *minutus*, *turgidus* (C), *varius*, *turgidus*	bark	not determined; *Phillipia* (A)	Elfin forest, Puerto Rico; Rwenzori, Uganda	Foerster (1971) and Pentecost (unpublished)
Gloeocapsa	*alpina*, *sanguinea*	bark	*Fraxinus excelsior* (A); not determined	England limestone pavement; Ceylon (Sri Lanka) forests	Pentecost (unpublished); Fritsch (1907a, b)
Merismopedia	*glauca*, *punctata*	?	?	Elfin forest, Puerto Rico	Foerster (1971)
Oscillatoriales					
Phormidium	*tenue*	?	?	Elfin forest, Puerto Rico	Foerster (1971)
Porphyrosiphon	*notarisii* (C)	bark	(A)	Common throughout Bangladesh	Nurul Islam (1972)
Pseudanabaena	sp.	bark	?	Atlantic rainforest, São Paulo State, Brazil	Branco et al. (2009)
Schizothrix	*cuspidata*	bark	?	Spain	Komárek and Anagnostidis (2005)
Nostocales					
Nostoc	*punctiforme*	bark	*Acer* (A)	Cumbria, UK	Pentecost (unpublished)
Scytonema	*millei*	bark	(A)	common in Bangladesh	Nurul Islam (1972)
Tolypothrix	*distorta*, *tenuis*	bark	*Acer pseudoplatanus* (A)	northern England, in seepage tracks down trunks	authors (unpublished)
Stigonematales					
Hapalosiphon	*intricatus*, *luteolus*, *welwitschii*	?	?	Elfin forest, Puerto Rico	Foerster (1971)
Stigonema	*hormoides* (C), *mesentericum* (C), *panniforme*	?	?	Elfin forest, Puerto Rico	Foerster (1971)

Where two or more taxa are recorded under one genus, locations and references follow in the same order
? indicates that the substratum is unknown

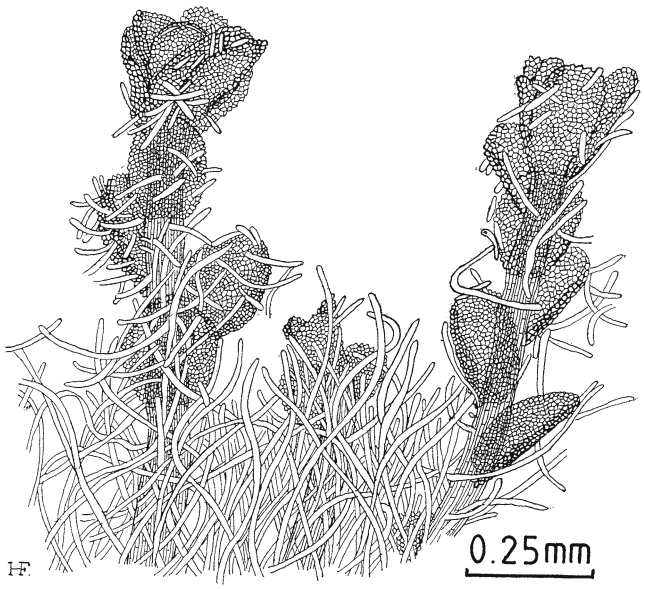

Fig. 10.11 *Tolypothrix* growing among bryophytes and using them as support (From Fritsch 1907a)

such cyanobacteria also benefit from the water retained by the capillarity of the moss stems and leaves. In some cases, cyanobacteria appear capable of competing effectively with bryophytes (Fig. 10.12) or even smothering them. Damp limestone in the UK frequently has crevice mosses such as *Tortella tortuosa* and *Barbula* spp. that become partially overgrown with subaerial *Nostoc* and *Gloeocapsa*. The former are often lichenized as *Collema* and *Leptogium* species. *Hypnum* and *Eurhynchium* on trees in the UK can be smothered by strata of *Phormidium corium* (A.P., unpublished). In the case of the biological soil crusts described in Chap. 12, succession typically progresses from an entirely cyanobacterial dominated community to one with mixed cyanobacteria and mosses. Here moss protonema and rhizoids intermingled with filamentous cyanobacteria also have a very important role in addition to the leafy moss shoots.

The most detailed studies have been conducted in north boreal and sub-arctic regions, where the focus of interest has been on N_2 fixation (Alexander and Billington 1986; Lennihan et al. 1994; DeLuca et al. 2002; Lagerström et al.

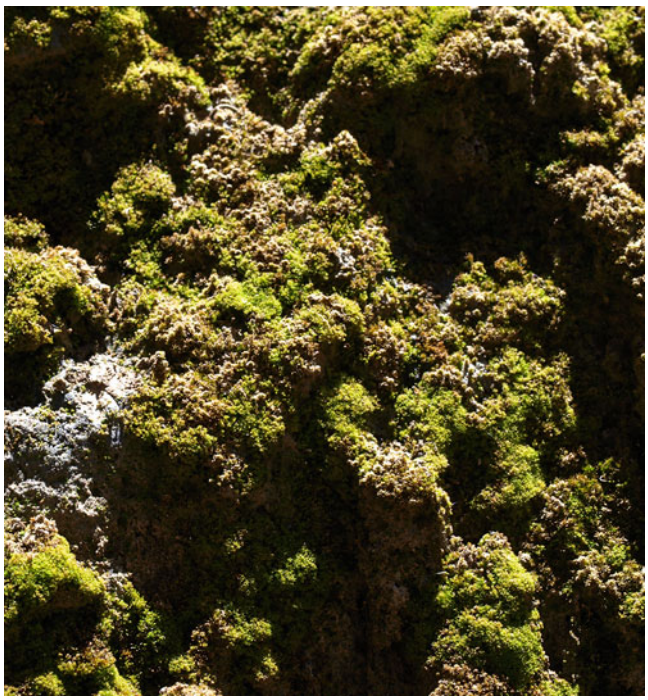

Fig. 10.12 Mixed cyanobacterial (including *Aphanothece* and *Schizothrix*) and moss community on shaded wall of Karasaili Canyon, SE. Turkey

2007; Markham 2009) although similar studies have also been reported from the Antarctic (Pandey et al. 1992). Here the situation is complicated by the fact that different cyanobacteria are in various types of association with particular moss species (see Gentili et al. 2005), ranging from true symbiosis (Chap. 23) to very close associations, where the relationship is probably influenced by the environment, to organisms which are simply epiphytes or in loose association. The moss *Pleurozium schreberi*, which is ubiquitous in boreal forests (Carleton and Read 1991), forms a close association with *Nostoc* sp. (DeLuca et al. 2002). *Hylocomium splendens*, another feather moss and also one of the commonest mosses in the world, was reported to be associated with *Nostoc* and *Stigonema* (Zackrisson et al. 2009), though this cyanobacterial association fixed about 50% less N seasonally than that with *Pleurozium*. The much divided shoots of these feather mosses provide an especially large surface for colonization, suggesting that the importance of cyanobacteria in their environments might have influenced their morphological evolution.

Late successional forests in northern Sweden showed higher rates of N_2 fixation and consistently higher numbers of cyanobacteria on moss shoots, but lower levels of available N, than early successional forests, which had previously been influenced by fire damage (Zackrisson et al. 2004, 2007). Transplantation of moss carpets resulted in a significant shift in presence and activity of cyanobacteria 1 year after initiation of the experiment responding to N fertility differences in early versus late successional forests. Early secondary succession forests yielded greater throughfall N deposition, which in turn decreased N_2 fixation by cyanobacteria associated with feather-moss carpets (DeLuca et al. 2007, 2008).

In the studies of Zackrisson et al., N_2-fixation rates were higher at northern latitudes (64–69°N) than further south and the authors comment that this is potentially related to differences in anthropogenic N deposition. The abundance of these mosses is not only much less in temperate regions, but N_2 fixation by the association tends to be reduced by atmospheric N deposition (Zackrisson et al. 2004). The effects of atmospheric N deposition were also suggested (Whitton 2000) to influence the absence in *Hapalosiphon* among *Sphagnum* communities of the Pennines in northern England compared with the frequency of *Hapalosiphon* in close association with *Sphagnum* at the edges of small pools in the Flow Country of N-E. Scotland. If so, it seems possible that cyanobacteria may have had a much greater role in the bryophyte abundant communities of temperate regions before the industrial revolution.

N_2 fixation by cyanobacteria associated with bryophytes was also important in the tree canopies of three pristine temperate rainforests in British Columbia, Canada (Lindo and Whiteley 2011). Although the cyanolichen *Lobaria* was abundant, the authors concluded that cyanobacteria associated with mosses contributed considerably more fixed N than cyanolichens. *Scytonema* was the main free-living cyanobacterium and its scytonemin-rich sheath may have contributed to its success here. Cyanobacterial density was considerably greater on epiphytic mosses than those on the forest floor and the authors suggested that this might result from N transfer from the epiphyllous communities to the ground underneath, since nutrient concentrations in suspended soil underlying epiphytic bryophytes are known to be higher than on forest floor soils at another western British Columbia site (Lindo and Winchester 2007). There are apparently no similar studies in tropical rain forests, but it should be possible to get some idea as to whether cyanobacteria are frequent among epiphyllous mosses by checking herbarium material.

Two of the three cyanobacteria colonizing *Pleurozium schreberi* in Swedish forests reported by Gentili et al. (2005) were isolated to permit study of the influence of temperature on N_2 fixation. Culture studies showed an obvious difference in the temperature optimum with that for *Calothrix* sp. being higher than that for *Nostoc* sp. However, these authors used the P-rich medium BG-11, so it would be of interest to repeat this study under a more natural environment, where the ambient P concentration might perhaps be three orders of magnitude lower. Although *Stigonema* and *Nostoc* were the co-dominants, the authors were unable to isolate the former. (Their photo shows an organism which could equally well be *Hapalosiphon*).

A further study (Ininbergs et al. 2011) on cyanobacteria associated with *Pleurozium shreberi* and *Hylocomium*

Table 10.5 Moss-associated cyanobacteria growing on limestone and granite, Cumbria, UK

	Limestone				Granite			
Moss	*Orthotrichum anomalum* (A)	*Tortula muralis* (A)	*Ctenidium molluscum* (P)	*Homalothecium sericeum* (P)	*Dicranum scoparium* (A)	*Andreaea rothii* (A)	*Hypnum cupressiforme* (P)	*Racomitrium fasciculare* (P)
Cyanobacterium								
Aphanocapsa fuscolutea		+				+		
Aphanocapsa muscicola	+							
Cyanothece aeruginosus			F					
Gloeocapsa alpina		+						
Gloeocapsa compacta	+	F	+	+	+			
Gloeocapsa punctata	+		+	+	+	+	+	+
Gloeocapsa sanguinea								+
Synechocystis sallensis	+			+				
Lyngbya aerugineo-caerulea		F		F				
Pseudanabaena minuta	+			F				
Pseudanabaena sp.						+		
Nostoc spp.	F	D	F	D				
Tolypothrix distorta	+							
Tolypothrix tenuis				F				

Dominant algae on granite were all Chlorophyta belonging to Chlorococcales, *Mesotaenium* and *Klebsormidium*
A Acrocarpous moss, *P* pleurocarpous moss, *D* dominant form, *F* frequent, + present

splendens based on *nifH* sequencing showed a high degree of host specificity. Two of the clusters of *nifH* sequences contained *nifH* phylotypes exclusively present on *H. splendens*. The authors suggested that moss species identity, but not extrinsic environmental factors, serves as the primary determinant of N_2-fixation cyanobacterial communities that inhabitat mosses. The reasons why the two mosses host different cyanobacterial communities are likely to involve the closeness of the association and differences in the physical or chemical microenvironments provided.

Other reports on cyanobacteria and bryophytes either deal with strict symbioses (Chap. 23) or are essentially floristic accounts. Gunale and Balakrishnan (1983) listed 16 cyanobacterial species growing on bryophytes in India. Among these, Chroococcales predominated with *Chroococcus turgidus* and *Gloeocapsa crepidinum* widespread together with *Nostoc punctiforme* and "*Nelliecarteria ramosa*" (probably *Microcoleus*); however, the flora was dominated by green algae. Foerster (1971) found 13 cyanobacterial species in squeezings from epiphytic bryophytes on Pico del Oeste, Puerto Rico, but they appeared to differ little from taxa growing directly on bark and again the Chroococcales were well represented. In Antarctica. Broady (1981) reported that cyanobacteria occurred epilithically often where water had trickled through bryophytes.

In order to understand further the relationship between cyanobacteria, bryophyte species and rock type, green stems and leaves of eight moss taxa were taken from limestone and granite rock surfaces in Cumbria, UK, and shaken well with a few mL of water (A.P., unpublished). The suspension was then examined with a light microscope and a semi-quantitative assessment undertaken based upon frequency of occurrence. Despite the crude approach, some marked differences were apparent between the two rock types supporting the bryophytes (Table 10.5). First, there appears to be a greater diversity of taxa among the limestone bryophytes with more evidence of filamentous taxa, particularly small colonies of *Nostoc* that were common in all of the samples. On granite, cyanobacteria were not found to be abundant and occurred as small colonies scattered among a much larger biomass of green algae, although *Gloeocapsa punctata* appeared to be reasonably well represented on both granite and limestone. Filamentous taxa were hardly represented on the granite and *Nostoc* was absent. In addition, the four cushion-forming acrocarpous mosses (A in Table 10.5) did not appear to differ in their cyanobacterium flora substantially from the mat-forming or pleurocarpous mosses (P). It therefore appears that the substratum type is more influential here than moss habit. This is probably due to the influence of dissolved substances originating from the rock through the processes of capillarity and diffusion within the moss cushions. Apart from the pigmented *Gloeocapsa* (*alpina*, *compacta*, *sanguinea*) that were usually present in small numbers, and probably originated from nearby rock surfaces, all taxa possessed hyaline sheaths and may have been protected from intense light by the moss stems and leaves.

10.4.7 Cyanolichens

The great majority of lichens occur in subaerial environments, although a few are aquatic or terricolous (Chap. 23). They are a successful group, particularly in water-stressed environments and have been estimated to dominate about 8% of the terrestrial surface (Ahmadjian 1995). In the cool temperate climate of the British Isles, about 10% of lichen taxa contain cyanobacteria. The proportion is almost exactly the same in the tropical Seychelles (Schumm and Aptroot 2010). Of the approximately 140 British cyanolichens (species whose major or sole photobiont is a cyanobacterium), foliose forms predominate (70%) with the remainder being crustose. There is also a group of chlorolichens containing small clusters of cyanobacteria, known as cephalodia. There are about 30 taxa of lichens with cephalodia in Britain, the majority of which are either shrubby or foliose (e.g. some *Peltigera* spp. and *Stereocaulon*).

The shrubby and foliose cyanolichens can also be categorised according to the spatial relationships of the two partners. In the homoiomerous genera, the cyanobacteria are dispersed throughout the body of the lichen (e.g. *Collema*, *Lempholemma*), while in the heteromerous group they are contained in a narrow stratum close to the surface (e.g. *Peltigera*, *Lobaria scrobiculata*) and enclosed by fungal hyphae. Some intermediate forms occur within the genus *Leptogium*. Only a few genera of cyanobacteria are lichenized, notably *Chroococcidiopsis*, *Gloeocapsa* (Chroococcales), *Nostoc*, *Scytonema* (Nostocales) and *Stigonema* (Stigonematales). There is a notable absence of lichenized Oscillatoriales, and lichens with cephalodia usually only contain *Nostoc* or *Stigonema*. The cyanolichens are distributed worldwide and most of the genera are cosmopolitan. They occupy a wide range of subaerial habitats, but base-rich sites are favoured by most species, at least in the British Isles (c. 60% total taxa).

The fungi of many lichens produce a huge range of extracellular compounds known as lichen substances. The cyanolichens however produce few of these substances, when compared with the chlorolichens and in many species no substances have been detected at all. Those that do possess them are heteromerous lichens such as *Lobaria*, *Nephroma* and *Peltigera*. Common substances are depsides and the hopane triterpenoids derived from mevalonic acid (Elix et al. 2008). Chlorolichens with cyanophyte cephalodia contain a wider range of lichen substances – gyrophoric acid for example, is often present. There is no consistent pattern between the occurrence of these substances and the presence of cyanobacteria, but many of them have been shown to possess antibiotic and antiviral properties and some have plant growth-regulating properties (Beckett et al. 2008). They might therefore find a role in the positioning and growth of the cyanobacterium within the lichen and provide some protection against pathogens and grazers.

Beckett et al. (2008) considered lichens to be classic stress tolerators and described a range of water- and radiation stress alleviators from them. They emphasize their poikilohydric nature and mention a range of mechanisms that lichens use to prevent damage from desiccation and intense solar radiation, such as structural modifications and pigment formation. One possible advantage for the cyanobacterium is increased availability of water. For example, some lichens are able to remove water from an atmosphere with a relative humidity less than 100% (Nash 2008). When the chlorophyll a content per unit area was compared for *Collema cristatum* (containing *Nostoc*) sampled from a limestone pavement in the UK with adjacent non-lichenized growths of *Gloeocapsa alpina/kuetzingiana*, that in the lichen population was much higher (65 mg m^{-2}) than in the free-living cyanobacterium (27 mg m^{-2}) (A.P., unpublished).

The lichenized cyanobacteria often grow in exposed situations subject to high solar irradiance. The homoiomerous species are usually dark brown in colour and contain dark brown extracellular pigments. Although these have not been studied critically, both produce UV-protective brown pigments, cyanobacterial scytonemin and fungal melanin. Unidentified brown pigments are also formed within the heteromerous group (e.g. *Peltigera rufescens*), but several lichen substances are also known to have light and UV-absorptive properties (Elix and Stocker-Wörgötter 2008; Ahmadjian 1993) and can be deposited in the upper cortex, thus shielding the cyanobacterium layer below. The stress caused by high irradiance can lead to the formation of reactive oxygen radicals with the potential to damage seriously both bionts (Beckett et al. 2008). In the case of *P. rufescens*, non-photochemical quenching has been observed as a photoprotective reaction (Lange et al. 1999). It results in heat dissipation through quenching via carotenoids within the cells. When the phycobiont and mycobiont of the chlorolichen *Cladonia* were cultured separately and exposed to stresses such as high irradiance, they performed less well than when combined as a lichen. This mutual association, with its exchange of metabolites and resulting structural modifications is thought to have contributed to the evolutionary success of these organisms (Kranner et al. 2005).

Associations are also apparent with other prokaryotes. For example, Lewin (2006) found a bacterium closely associated with an isolate of *Gloeocapsa* that he suspected was utilizing EPS material, while at the same time being offered some protection from desiccation.

10.4.8 Mine Spoil

There are a sufficiently large number of records of cyanobacteria growing in metal-rich conditions that it seems probable that they can produce strains tolerant to at least most metals. However, it is much easier to quantify the relationship when

Fig. 10.13 Intermittently moist Zn-rich surfaces at Elvins tailings, Missouri, covered with mixed community of *Plectonema gracillimum* (*Leptolyngbya gracillima*) and predominantly the protonemal stage of the moss *Dicranella*. The cyanobacterial sheaths develop a *brown colour* where there is a permanent trickle of water (with 16 mg L^{-1} Zn)

the metals are in solution. For instance, Whitton and Diaz (1980) reported that in a study of 1,614 stream and pond sites, 20% cyanobacterial taxa in water with ≤0.1 mg L^{-1} Zn, also occurred in water in the range >10 ≤ 100 mg L^{-1} Zn. A community of *Plectonema gracillimum* (*Leptolyngbya gracillima*) and protonema of several species of the moss *Dicranella* has been found on old Zn-Pb spoil heaps in a number of countries, including France, Germany, Italy, UK and USA (B.A.W. records). This occurs both on sediments which are dry for much of the time and in small streams, such as the described by Whitton et al. (1981), where the Zn reached 16 mg L^{-1} (Fig. 10.13). The relative proportion of moss increases as the community develops and in less stressed environments leafy shoots of the moss become increasingly predominant. The much wider filaments of *Lyngbya aestuarii* occurred on lead mine spoil at Cat Bells, Cumbria (A.P., unpublished).

Among reports of cyanobacteria on particular types of rock, Anagnostidis and Roussomoustaki (1988) found *Gloeocapsa kuetzingiana* on iron-nickel ores in Greece, together with *Phormidium subfuscum* and *Leptolyngbya boryana*. *L. boryana*, *L. foveolarum* and *Chlorogloea microcystoides* occurred on limonite and ochre. Cyanobacteria were absent from Cu-containing ores such as azurite and malachite. Cyanobacteria were also absent on the iron rails studied by Schlichting (1975), although green algae were present. However, *Gloeocapsa sanguinea* was found on calcareous haematite ore at Ascomb, Cumbria (A.P., unpublished).

The cyanolichen genus *Peltigera* and the chlorolichen genus *Stereocaulon* with *Stigonema* cephalodia often colonize rocks with high heavy metal contents such as copper and lead mine waste. It is unclear whether sequestration of metal ions by *Stereocaulon* reduces the metal concentrations in the vicinity of the cyanobacterium or whether the latter is equally adapted to the metal as the fungus.

10.4.9 Snow and Ice

At least in Eurasia and North America, Kol's (1968) records indicate that cyanobacteria are a minor component of the kryoflora, with only a few species in contrast to 30 species of green algae. All the cyanobacteria were Chroococcales and included *Aphanocapsa nivalis*, *Rhabdogloea hungarica* and *Rhabdoderma transsylvanica*. They sometimes impart a bluish tint to snow, but also occur in 'black snow' or as part of a mixed community with coccoid Chlorophyceae. While many species of *Aphanocapsa* occur in other subaerial habitats, *Rhabdogloea* and *Rhabdoderma* are usually found in the plankton. Kol did not note sheath pigmentation, despite the high UV radiation often experienced in snowfields that has led to protective pigments being produced by some of the kryophilic green algae such as *Chlamydomonas nivalis*. This suggests that their occurrence may have been opportunistic, resulting in a brief period of growth during periods of snow cover and thus a temporary endolith or under conditions where surface irradiance was low. T he common subaerial *Gloeocapsa sanguinea* was found in melting snow by Sheath et al. (1996), and may also represent chance colonization of a species with sheath pigmentation. Dust accumulations on snowfields, known as snow cryoconites have also been found associated with cyanobacteria (Takeuchi et al. 2001). The radiation-absorbing qualities of dust may be advantageous to colonization and also provide a source of P and other nutrients.

10.4.10 Man-Made Structures

Unsightly growths of cyanobacteria on the roofs, and especially, the walls of buildings have long been recognized (Wee and Lee 1980; Strzelczyk 1981; Lewin 2006). Walls containing calcareous rock such as limestone and concrete or those with mortar courses, plaster or whitewashed with calcium carbonate are particularly affected. The cyanobacteria may impart either a darkening of the surface without any obvious formation of colonies or more evident streaks, blotches or lines resulting from rain tracks or occasionally trickles and drips. They can give a general impression of neglect, even though in tropical situations, walls may have been whitewashed the previous year. Species of *Gloeocapsa* are often responsible, especially in temperate regions, but a number of other cyanobacteria are known to colonize buildings, differing little from those found on steep calcareous rock surfaces in the same region.

Over 20 taxa of cyanobacteria were recorded by Schlichting (1975) from buildings in Ireland, parts of which are subject to frequent rainfall; the most frequent genera were *Chroococcus*, *Gloeocapsa*, *Oscillatoria* and *Schizothrix*. *Chroococcidiopsis* and *Gloeocapsa* were the dominants of colonial crusts in Gimhae, Korea, with the former on terrestrial surfaces that remained wet for long periods, while the latter were more common on surfaces moist for only brief periods (Tripathi et al. 2007). The moist environment for *Chroococcidiopsis* is sufficiently different from most other reports for this genus (Chap. 12), that it merits further taxonomic study. *Aphanocapsa* and *Chroococcus* were the next most frequent genera.

As the cyanobacterial flora of historically important monuments is described in Chap. 11, only the study on ancient marbles of the Parthenon in Greece by Anagnostidis et al. (1983) is mentioned here, because it was one of the first to provide a detailed and accurate account. Cyanobacteria were the most important phototrophs and included 14 taxa, mostly Chroococcales (*Chroococcus, Gloeocapsa* and *Myxosarcina* spp.). These were mostly epilithic and chasmoendolithic and were suspected of being responsible for some of the biodeterioration of the marble. John (1988) listed more than 50 taxa from buildings, mainly Chroococcales and the Oscillatoriales. Most surfaces sampled were stonework, but other artificial surfaces were also affected. Asphalt shingles, used widely for buildings in North America, are often discoloured with cyanobacteria (Fig. 18.2). In a survey of sites in Canada and USA, Brook (1968) found that green algae colonized before the cyanobacteria; *Gloeocapsa magma* and *G. rupestris* eventually took over, blackening the surfaces, particularly on north- and west-facing roofs. Cyanobacterial success on asphalt tiles has subsequently been shown to be encouraged by particles of lime included in the asphalt mixture. A number of authors have described the cyanobacteria on roof tiles in India e.g. Dan et al. (1982). Although *Gloeocapsa sanguinea* is best known from rock surfaces, it can form brown streaks on neglected tarmac roads in Wales, UK. Geitler (1932) reported *Lyngbya gracillimum* on a stained glass window. The frescoe deterioration associated with *Lyngbya* and *Plectonema* spp. under low illumination within Roman buildings reported by Caiola et al. (1987) has subsequently been studied in some detail (Chap. 11).

The vertical black streaks on buildings in moister areas of the tropics and subtropics are often dominated by *Tolypothrix* rather than Chroococcales, but the reasons for its greater success here are not entirely clear. In some cases the lime white wash may be more P-rich in the tropics, as when obtained from ground coral, and thus favour a N_2-fixer. However, this is unlikely to be the main explanation, as eukaryotic algae show a similar morphological difference, with branching Trentepohliales dominating many surfaces in the tropics, as opposed to coccoid green algae in temperate regions. Such growths are often considered sufficiently visually offensive to require removal by scrubbing or a fresh coat of lime whitewash; Singapore has become well-known for making this a legal requirement. No permanent solution to this problem is known, but paints containing algicides have proved effective over periods of a few years (Schlichting 1975; Lewin 2006). A colourimetric method for estimating the effect of such algicides is described by Sanmartín et al. (2010).

The problem becomes more serious when there is marked erosion of a limestone surface. Pleurocapsales growths on limestone sculptures and walls in Israel accelerated the weathering rate to about 0.5 mm/100 years (Danin and Canova 1990). Danin (1993) investigated the processes involved on marble at the Temple of Apollo at Didim, Turkey. Two of the three processes involved were due directly to cyanobacteria: Surface exfoliation was associated with Pleurocapsales in fissures and pits formed by cyanobacteria living in circular patches. The removal of marble crystals led to the creation of depressions favourable to the growth a microbial community, again dominated by cyanobacteria. Control with algicides becomes more of a challenge when the problem growths are endolithic, such as in the marble fountains of the Alhambra, Granada (Bolivar and Sanchez-Castillo 1997). These are related studies are discussed further in Chap. 11.

Occasionally, discolouration is encouraged on surfaces. Abandoned quarries, for example, can leave ugly scars on the landscape and attempts are often made to camouflage these by encouraging algal growth by spraying the rock with diluted manure or sewage sludge to speed the colonization process.

10.5 Temporal Changes

Colonization on artificial surfaces often shows an initial lag phase partially owing to the high pH of materials such as concrete and mortar. Wee and Lee (1980) observed growth of

Table 10.6 Examples of biomass measurements of subaerial cyanobacterial communities as cellular biovolume per unit area

Substratum	Location	Dominant species	Cellular biovolume ($mm^3\ cm^{-2}$ [a])	References
Limestone tintenstriche	Malham Cove, England	*Gloeocapsa alpina*, *G. kuetzingiana*	0.32	Pentecost (1982)
Quartzite pebbles	Ben Eighe, Scotland	*Gloeocapsa magma*	0.24	A.P. (unpublished)
Basalt	Glen Coe, Scotland	*Gloeocapsa sanguinea*	0.07	A.P. (unpublished)
Bark of *Fraxinus excelsior*	Cumbria, England	*Gloeocapsa compacta*, *G. sanguinea*, *G. punctata*	0.14	A.P. (unpublished)
Limestone scree	Grey Gill, N. Yorkshire	*Gloeocapsa alpina, G. kuetzingiana*	0.7	A.P. (unpublished)
Mat in warm effluent	Taffs Wells, Wales	*Phormidium ambiguum*	4.1	Pentecost and Whitton (2000)
Waterbloom	L. Lucerne, Switzerland	*Phormidium ambiguum*, *Oscillatoria rubescens*	2.4	Zimmermann (1969)

A waterbloom is included for comparison
[a]Excludes sheath/EPS

algae 1 year after the completion or painting of buildings in Singapore. Although the first phototroph to colonize was often the green alga *Trentepohlia*, this was later overtaken by cyanobacteria. Likewise, fresh limestone surfaces resulting from quarrying appear to take several years before they acquire an obvious algal cover (John 1988). In some cases freshly exposed surfaces may require a period of weathering, facilitating ingress of water into the surface layers of rock. The rate of colonization must also be influenced by the availability of propagules transported to the site by air, water or biological means such as invertebrates and birds.

Gloeocapsa took several years to colonize calcareous walls in a study by Garty (1990) in Canada. At Malham Cove, England, where coccoid cyanobacteria formed dark tintenstriche on an 80-m high south-facing limestone cliff rarely exposed to water, the effect was studied (Pentecost 1982) of cleaning a streak consisting of *Chlorogloea microcystoides* and *Gloeocapsa* spp. After observation for 10 years the biomass was barely 10% of an adjacent uncleared control area. In the case of a tintenstriche receiving regular wetting (approx. 250 days per year), the biomass of a *Schizothrix* community showed complete recovery within 1 year. Similarly, substantial colonization of limestone was noted after a period of 1 year on Aldabra Atoll (Viles 1988) on frequently wetted sites, although some drier sites were still not fully recolonized after 16 years (H.A. Viles, personal communication 1999). These demonstrate the importance of water availability and the slow rates of growth and colonization where water is limited. This applies equally to cool temperate and tropical subaerial environments, but it is also evident that when water is available, growth is far more rapid at the lower latitudes in response to higher temperatures.

Cellular biovolume estimates per unit area are a useful means of comparing cyanobacterial biomass in different types of community. Estimates for the subaerial environment (Table 10.6) show a biovolume range from 0.07 to 0.7 $mm^3\ cm^{-2}$. Comparative values for freshwater plankton can be estimated from Reynolds (1984) by combining his estimate of the chlorophyll a contents of cyanobacterial populations (mean of 5.7×10^{-15} g μm^{-3}) with theoretical maximum cover value of chlorophyll-a (ca. 300 mg m^{-2}), which result in a theoretical maximum cellular biovolume of about 5 $mm^3\ cm^{-2}$. The values for subaerial environments are clearly much lower than those for waterblooms and also dense mats (Chap. 9), reflecting the fact that they are stressed environments limited by availability of water. Changes in biomass per unit area can also be followed at a particular site based on chlorophyll a, provided the phototroph community is almost entirely cyanobacterial. A study (Pentecost 1992) of a tintenstriche community at Malham Cove, Yorkshire, UK, which consisted mostly of Chroococcales, showed an initial chlorophyll a content of c 30 mg m^{-2}; after an area had been cleaned, the value was less than 2 mg m^{-2} after 1 year and still only 2.9 mg m^{-2} after 10 years. The methods for comparing cyanobacterial biomass in more complex terrestrial communities are discussed in Chap. 12.

Possible genetic and community changes of subaerial cyanobacteria in response to geological and historical periods of time have been mentioned briefly in this review, but there have apparently been no critical studies on past events similar to those done on lakes. However, the practical use of inoculation procedures (Chap. 12) shows the considerable potential for modifying communities if the environment is controlled in various ways, at least during the initial period following inoculation. Many other changes are probably occurring in response to climatic changes and atmospheric pollution, but they only gain attention when there are visually obvious effects, such as unsightly growths on walls.

10.6 Conclusions

Although subaerial cyanobacteria and algae have been studied for at least a century and there have been many accounts of their distribution and the factors influencing this, understanding of their ecology on rock surfaces and buildings is still

rather superficial. This contrasts with the increasing insight to the ecology of phototrophs in underground monuments receiving illumination and the biological soil crusts of semi-desert regions. Part of the problem has been the challenge of providing consistent names to organisms with few obvious morphological features, but detailed molecular studies such as those which have been applied to desert endolith communities should help to solve this. In turn it should be possible to assess much more critically the changes which have occurred and are now occurring on rock surfaces in different parts of the world as a result of atmospheric pollution and climatic changes.

References

Ahmadjian V (1993) The lichen symbiosis. Wiley, New York, 250 pp

Ahmadjian V (1995) Lichens are more important than you think. BioScience 45:123–124

Alexander V, Billington M (1986) Nitrogen fixation in the Alaskan taiga. In: Vleve V (ed) Forest ecosystems in the Alaskan Taiga. Springer, New York, pp 112–121

Al-Thukair AA, Golubic S (1991) New endolithic cyanobacteria from the Arabian Gulf. 1. *Hyella immanis* sp. nov. J Phycol 27:766–780

Anagnostidis K, Komárek J (1998) Cyanoprokaryota 1. Teil: Chroococcales. Süsswasserflora von Mitteleuropa. Gustav Fischer, Jena

Anagnostidis K, Roussomoustaki M (1988) Cyanophytes from metal-burdened substrates. Arch Hydrobiol Suppl 80; Algol Stud 50–53: 561–564

Anagnostidis K, Economou-Amilli A, Roussomoustakaki M (1983) Epilithic and chasmoendolithic microflora (Cyanophyta, Bacillariophyta) from marbles of the Parthenon (Acropolis-Athens, Greece). Nova Hedwigia 38:227–287

Anon (2005) http://www.EngineeringToolBox.com/

Badger MA, Hanson D, Price GD (2002) Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. Funct Plant Biol 29:161–173

Beckett RP, Kranner I, Minibayeva FV (2008) Stress physiology and symbiosis. In: Nash TH III (ed) Lichen biology, 2nd edn. Cambridge University Press, Cambridge, pp 136–153, 286 pp

Bell RA, Athey PV, Sommerfeld MR (1988) Distribution of endolithic algae on the Colorado Plateau of northern Arizona. Southwest Nat 33:315–322

Bennett P, Siegel DI (1987) Increased solubility of quartz in water due to complexing organic compounds. Nature 326:684–687

Bergman B, Osborne B (2002) The *Gunnera-Nostoc* symbiosis. Biol Environ 102B:35–39

Bolivar FC, Sanchez-Castillo PM (1997) Biomineralization processes in the fountains of the Alhambara, Granada, Spain. Biodeterior Biodegrad 40:205–215

Borzi A (1892) Alghe d'acqua dolce delle Papuasia, raccolte su cranii umani dissepolti. Nuov Notarisia (for 1892) 3:35–53

Branco LHZ, Hoffmann L, Teiveira JP, de Morais-Filho JC (2009) Aerophytic cyanoprokaryotes from the Atlantic rainforest region of São Paulo State, Brazil: Chroococcales and Oscillatoriales. Cryptogam Algol 30:135–152

Broady PA (1981) Ecological and taxonomic observations on subaerial epilithic algae from Princess Elizabeth Land and MacRobertson Land, Antarctica. Br Phycol J 16:257–266

Brook AJ (1968) The discoloration of roofs in the United States and Canada by algae. J Phycol 4:250

Brook GA, Folkoff ME, Box EO (1983) A world model of soil carbon dioxide. Earth Surf Proc Landf 8:79–88

Büdel B, Bendix J, Bicker FR, Green TGA (2008) Dewfall as a water source frequently activates the endolithic cyanobacterial communities in the granites of Taylor Valley, Antarctica. J Phycol 44:1415–1424

Büdel B, Schulz B, Reichenberger H, Fritz B, Green TGA (2009) Cryptoendolithic cyanobacteria from calcite marble rock ridges, Taylor Valley, Antarctica. Algol Stud 129:61–69

Caiola MG, Forni C, Albertano P (1987) Characterization of the algal flora growing on ancient Roman frescoes. Phycologia 26:387–396

Chacón E, Berrendero E, Garcia-Pichel F (2006) Biogeological signatures of microboring cyanobacterial communities in marine carbonates from Cabo Rojo, Puerto Rico. Sediment Geol 185:215–228

Chapman P (1993) Caves and cave life, New naturalist series. Harper Collins, London, 220 pp

Claus G (1962) Data on the ecology of algae of Peace Cave in Hungary. Nova Hedwigia 21:55–79

Couté A, Bury E (1988) Ultrastructure d'une cyanophyceé aerienne calcifeé cavernicole: *Scytonema julianum* (Frank) Richter (Hormogoneophyceae, Nostocales, Scytonemataceae). Hydrobiologia 160:219–240

Cox G, Benson D, Dwarte DM (1981) Ultrastructure of a cave wall cyanophyte, *Gloeocapsa* NS4. Arch Microbiol 130:165–174

Cox G, James JM, Leggett KEA, Osborne RAL (1989) Cyanobacterially deposited speleothems: subaerial stromatolites. Geomicrobiol J 7:245–252

Dan TK, Sreedharan VP, Patel M, Rothatgi FK (1982) The mechanism of blackening of tile surfaces. Int Biodeterior Bull 18:99–104

Danin A (1993) Biogenic weathering of marble monuments in Didim, Turkey, and in Traian Column, Rome. Water Sci Technol 27:557–563

Danin A, Canova G (1990) Deterioration of limestone walls in Jerusalem and marble monuments in Rome caused by cyanobacteria and cyanophilous lichens. Int Biodeterior 26:397–417

Danin A, Gerson R, Marton K, Garty J (1982) Patterns of limestone and dolomite weathering by lichens and blue-green algae and their paleoclimatic significance. Palaeogeogr Palaeoclimatol Palaeoecol 37:211–233

Darlington A (1981) Ecology of walls. Heinemann Educational Books, London

Davis JS, Rands DG (1982) Lime incrusting *Hapalosiphon intricatus* (Cyanophyceae) and phosphate availability in a Florida cave. Schweiz Z Hydrol 44:289–294

de Caire GZ, de Cano MS, de Mulé MCZ, Palma RM, Colombo K (1997) Exopolysaccharide of *Nostoc muscorum* (Cyanobacteria) in the aggregation of soil particles. J Appl Phycol 9:249–253

DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forest. Nature 419:917–920

DeLuca TH, Zackrisson O, Gentili F, Sellstedt A, Nilsson MC (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Ecologia 152(1):121–130

DeLuca TH, Zackrisson O, Gundale MJ, Nilsson M-C (2008) Ecosystem feedbacks and nitrogen fixation in boreal forests. Science 320:1181

Elix JA, Stocker-Wörgötter E (2008) Biochemistry and secondary metabolites. In: Nash TH III (ed) Lichen biology, 2nd edn. Cambridge University Press, Cambridge, pp 106–135, 286 pp

Ercegović A (1925) La végétation des lithophytes sur les calcaires et les dolomites en Croatie. Acta Bot Croat 1:64–114

Ercegović A (1932) Études écologiques et sociologiques des Cyanophycées lithophytes de la côte Yougoslave de l'Adriatique. Bull Int Acad Yougosl Sci Beaux Arts 26:33–56

Fjerdingstad E (1957) A lime incrusting community of a Danish well. Rev Algol 4:246–248

Fjerdingstad E (1965) The algal flora of some 'Tintenstriche' in the 'Alpes Maritimes' (France). Schweiz Z Hydrol 27:167–171

Fletcher A (1973) The ecology of maritime (supralittoral) lichens of some rocky shores of Anglesey. Lichenologist 5:401–422
Foerster JW (1971) The ecology of an elfin forest in Puerto Rico, 14. The algae of Pico del Oeste. J Arnold Arbor 52:86–109
Friedmann EI, Larock PA, Brunson JO (1980) Adenosine triphosphate (ATP), chlorophyll, and organic nitrogen in endolithic microbial communities and adjacent soils in the dry valleys of southern Victoria Land. Antarct J US 15:164–167
Fritsch FE (1907a) The subaerial and freshwater algal flora of the tropics. Ann Bot 21:235–275
Fritsch FE (1907b) A general consideration of the subaerial and freshwater algal flora of Ceylon. A contribution to the study of tropical algal ecology. Part 1. Subaerial algae and algae of the inland fresh waters. Proc R Soc B 79:197–254
Fritsch FE, Rich F (1924) Contributions to our knowledge of the freshwater algae of Africa 4. Trans R Soc S Afr 11:297–398
Gademann K, Portmann C (2008) Secondary metabolites from cyanobacteria: complex structures and powerful bioactivities. Curr Org Chem 12:326–341
Garcia-Pichel F, Ramírez-Reinat E, Gao Q (2010) Microbial excavation of solid carbonates powered by P-type ATPase-mediated transcellular Ca^{++} transport. Proc Natl Acad Sci USA 107(50):21749–21754
Garty J (1990) Influence of epilithic microorganisms on the surface temperature of building walls. Can J Bot 68:1349–1353
Geitler L (1932) Cyanophyceae. In: Rabenhorst L (ed) Kryptogamen-Flora von Deutschland, Österreich und der Schweiz 14. Akademische Verlagsgesellschaft, Leipzig, p 1139
Gentili F, Nilsson M-C, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular diversity of feather moss associative N_2-fixing cyanobacteria. J Exp Bot 56:3121–3127
Golubić S (1967a) Die Algenvegetation an Sandsteinfelsen Ost-Venezuelas (Cumaná). Int Rev Ges Hydrobiol 52:693–699
Golubić S (1967b) Algenvegetation der Felsen. In: Elster H-J, Ohle W (eds) Die Binnengewasser 23. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart
Golubic S, Friedmann I, Schneider J (1981) The lithobiontic niche, with special reference to microorganisms. J Sediment Petrol 51:475–478
Gorbushina AA (2007) Life on the rocks. Environ Microbiol 9(7):1613–1631
Gunale VR, Balakrishnan MS (1983) Studies on the subaerial algal flora epiphytic on bryophytes. Indian J Bot 6:202–209
Hall K, Lindgren BS, Jackson P (2005) Rock albedo and monitoring of thermal conditions in respect of weathering: some expected and some unexpected results. Earth Surf Proc Landf 30:801–811
Herrera A, Cockell CS, Self S, Blaxter M, Reitner J, Thorsteinsson T, Arp G, Drose W, Tindle AG (2009) A cryptoendolithic community in volcanic glass. Astrobiology 9:369–381
Hirose H, Akyiama M (1967) A review of aerial and soil algae III. Bull Jpn Soc Phycol 15:107–118
Hoffmann L (1986) Algues bleues aériennes et subaériennes du Grand-Duché de Luxembourg. Bull Jard Bot Natl Belg 56:77–127
Hoffmann L (1989) Algae of terrestrial habitats. Bot Rev 55:77–105
Ininbergs K, Bay G, Rasmussen U, Ward DA, Nilsson M-C (2011) Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. New Phytol. doi:10.1111/j.1469-8137.2011.03809.x (11 pp)
Issa OM, Défarge C, Le Bissonais Y, Marin B, Duval O, Bruand A, S'Acqui LP, Nordenberg S, Annerman M (2007) Effects of the inoculation of cyanobacteria on the microstructure and the structural stability of a tropical soil. Plant Soil 290:209–219
Jaag O (1945) Untersuchungen über die Vegetation und Biologie des näckten Gestein in den Alpen und im schweizerischen Mittelland. Schweiz Natur Ges 9:1–560 (For *Gloeocapsa*, see pp 180–221)
John DM (1988) Algal growths on buildings: a general review and methods of treatment. Biodeterior Abstr 2:81–102
Kol E (1968) Kryobiologie: Biologie und Limnologie der Schnees und Eises. I. Kryovegetation. In: Elster HJ, Ohle W (eds) Die Binnengewasser XXIV. Schweizerbart'sche, Stuttgart, 216 pp
Komárek J, Anagnostidis K (2005) Cyanoprokaryota: 2.Teil/Part 2. Oscillatoriales. In: Büdel B, Krienitz L, Gärtner G, Schagerl M (eds) Süßwasserflora von Mitteleuropa 19(2). Spektrum/Elsevier, Heidelberg, 759 pp (Reprinted by Spektrum/Springer in 2008)
Kranner I, Cram WJ, Zorn M (2005) Antioxidants and photoprotection in a lichen as compared with its isolated symbiotic partners. Proc Natl Acad Sci USA 102:3141–3146
Lagerström A, Nilsson M-C, Zackrisson O, Wardle DA (2007) Ecosystem input of nitrogen through biological fixation in feather mosses during ecosystem retrogression. Funct Ecol 21:1027–1033
Lange OL, Leisner JMR, Bilger W (1999) Chlorophyll fluorescence characteristics of the cyanobacterial lichen *Peltigera rufescens* under field conditions. II Diel and annual distribution of metabolic activity and possible mechanisms to avoid photoinhibition. Flora 194:413–430
Leclerc JC, Couté A, Dupuy P (1983) Le climat annuel de deux grottes et d'une église du Poitou, où vivant des colonies pures d'algues sciaphiles. Crypt Algol 4:1–19
Lefévre M (1974) La 'Maladie verte' de Lascaux. Stud Conserv 19:126–156
Lennihan R, Chapin SDM, Dickson LG (1994) Nitrogen fixation and photosynthesis in high arctic forms of *Nostoc commune*. Can J Bot 72:940–945
Lewin RA (2006) Black algae. J Appl Phycol 18:699–702
Lewin RA, Farnsworth PA, Yamanaka G (1981) The algae of green polar bears. Phycologia 20:303–314
Liermann LJ, Kalinowski BE, Brantley SL, Ferry JG (2000) Role of bacterial siderophores in dissolution of hornblende. Geochim Cosmochim Acta 64:587–602
Lindo Z, Whiteley JA (2011) Old trees contribute bio-available nitrogen through canopy bryophytes. Plant Soil 342:141–148
Lindo Z, Winchester NN (2007) Oribatid mite communities and foliar litter decomposition in canopy suspended soils and forest floor habitats of western redcedar forest, Vancouver Island, Canada. Soil Biol Biochem 39:2957–2966
Littler MM, Littler DS, Blair S, Norris JN (1985) Deepest known plant life discovered on an uncharted seamount. Science 227: 57–59
Lundberg J, McFarlane DA (2011) Subaerial freshwater phosphatic stromatolites in Deer Cave, Sarawak – a unique geobiological cave formation. Geomorphology 128:57–72
Mandelbrot BB (1983) The fractal geometry of nature, 2nd edn. WH Freeman & Co., New York
Markham J (2009) Variations in moss-associated nitrogen fixation in boreal forest stands. Oecologia 161:353–359
Martınez A, Asencio AD (2009) Distribution of cyanobacteria at the Gelada Cave (Spain) by physical parameters. J Cave Karst Stud 72:11–20
Meeks JC, Elhai J, Thiel T, Potts M, Larimer FW, Lamerdin J, Predki P, Atlas R (2001) An overview of the genome of *Nostoc punctiforme*, a multicellular symbiotic cyanobacterium. Photosynth Res 70: 85–106
Mikhailyuk TI (2008) Terrestrial lithophilic algae in a granite canyon of the Teteriv River (Ukraine). Biologia 63(6):824–830
Mulec J, Kosi G, Vrhovsek D (2008) Characterization of cave aerophytic algal communities and effects of irradiance levels on production of pigments. J Cave Karst Stud 70:3–12
Nash TH III (2008) Introduction. In: Nash TH III (ed) Lichen biology, 2nd edn. Cambridge University Press, Cambridge, UK, p 286
Neustupa J, Škaloud P (2010) Diversity of subaerial algae and cyanobacteria growing on bark and wood in the lowland tropical forest of Singapore. Plant Ecol Evol 143:51–62

Nienow JA, Friedmann EI (1993) Terrestrial lithophytic (rock) communities. In: Friedmann EI, Thistle AB (eds) Antarctic microbiology. Wiley, New York, pp 343–412
Nurul Islam AKM (1972) Subaerial algae of Bangladesh. Bangladesh J Bot 1:13–64
Olsson-Francis K, de la Torre R, Cockell CS (2010) Isolation of novel extreme-tolerant cyanobacteria from a rock-dwelling microbial community by using exposure to low earth orbit. Appl Environ Microbiol 76(7):2115–2121
Omelon CR, Pollard WH, Ferris FG (2006) Environmental controls on microbial colonization of high Arctic cryptoendolithic habitats. Polar Biol 30:3019–3029
Onek LA, Smith RJ (1992) Calmodulin and calcium mediated regulation of prokaryotes. J Gen Microbiol 138:1039–1049
Pandey KD, Kashyap AK, Gupta RK (1992) Nitrogen fixation by cyanobacteria associated with moss communities in Schirmacher oasis, Antarctica. Isr J Bot 41:187–198
Pentecost A (1978) Blue-green algae and freshwater carbonate deposits. Proc R Soc Lond B 200:43–61
Pentecost A (1982) A quantitative study of calcareous and Tintenstriche algae from the Malham district, Northern England. Br Phycol J 17:443–456
Pentecost A (1992) A note on the colonisation of limestone rocks by cyanobacteria. Arch Hydrobiol 124:167–172
Pentecost A (1993) Field relationships between scytonemin density, growth and irradiance in cyanobacteria occurring in low illumination regimes. Microb Ecol 26:101–110
Pentecost A (1998) The significance of calcite (travertine) formation by algae in a moss-dominated travertine from Matlock Bath, England. Arch Hydrobiol 143:487–509
Pentecost A (2010) Bark surface area in English woodlands - how much is there and how much do we sample? Bull Br Lichen Soc 107:35–43
Pentecost A (2011) Some 'Lamp Floras' from show caves in Northern England. Cave Karst Sci (in press)
Pentecost A, Rose F (1985) Changes in the cryptogam flora of the Wealden sandrocks, 1688–1984. Bot J Linn Soc 90:217–230
Pentecost A, Whitton BA (2000) Limestones. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer, Dordrecht, pp 258–279, 669 pp
Pentecost A, Zhang Z (2004) A note on the distribution of plants in Scoska Cave, North Yorkshire, United Kingdom, and their relationship to light intensity. J Cave Karst Sci 31:119–122
Petersen JB (1915) Studier over Danske aërofile alger. K Danske Vidensk Selsk Ser 7. 12:272–379
Petersen JB (1928) The aerial algae of Iceland. In: Rosenvigne LK, Warming E (eds) Botany of Iceland 2. J. Frimodt, Copenhagen, pp 327–447
Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755–805
Poulickova A, Hasler P (2007) Aerophytic diatoms from caves of Central Moravia (Czech Republic). Preslia 79:185–204
Price GD, Badger MR (2002) Advances in understanding aquatic photosynthetic organisms utilize sources of dissolved inorganic carbon for CO_2 fixation. Funct Plant Biol 29:117–121
Price GD, Maeda S, Omada T, Badger MR (2002) Modes of active inorganic carbon uptake in the cyanobacterium *Synechoccocus* sp. PCC7942. Funct Plant Biol 29:131–149
Proteau PJ, Gerwick WH, Garcia-Pichel F, Castenholz RW (1993) Structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. Experientia 49:825–829
Raven J, Cockell CS (2006) Influence on photosynthesis of starlight, moonlight, planetlight and light pollution (reflections on PAR in the universe). Astrobiology 6:668–675
Reynolds CS (1984) The ecology of freshwater Phytoplankton. Cambridge University Press, Cambridge, UK
Sanmartín P, Villa F, Silva B, Cappitelli F, Prieto B (2010) Color measurements as a reliable method for estimating degradation to pigments. Biodegradation 22(4):763–771
Schlichting HE Jr (1975) Some subaerial algae from Ireland. Br Phycol J 10:257–261
Schumm F, Aptroot A (2010) Seychelles lichen guide. Beck OHG, Süssen
Shaw E, Hill DR, Brittain N, Wright DJ, Tauber U, Marand H, Helm RF, Potts M (2003) Unusual water flux in the extracellular polysaccharide of the cyanobacterium *Nostoc commune*. Appl Environ Microbiol 69(9):5679–5684
Sheath RG, Vis ML, Hambrook JA, Kole KM (1996) Tundra stream macro-algae of North America: composition, distribution and physiological adaptations. Hydrobiologia 336:67–82
Strzelczyk AB (1981) Stone. In: Rose AH (ed) Microbial biodeterioration. Economic microbiol 6. Academic, London, pp 61–80
Stumm W, Morgan JJ (1996) Aquatic chemistry – chemical equilibria and rates in natural waters, 3rd edn. Wiley Interscience, New York/London
Suutari M, Majaneva M, Fewer DP, Voirin B, Aiello A, Friedl T, Chiarello AG, Blomster J (2010) Molecular evidence for a diverse green algal community growing in the hair of sloths and a specific association with *Trichophilus welckeri* (Chlorophyta, Ulvophyceae). BMC Evol Biol 10:86. doi:10.1186/1471-2148-10-86
Takeuchi N, Kohshima S, Seko K (2001) Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: a granular algal mat growing on the glacier. Arct Antarct Alp Res 33:115–122
Tian Y, He F (1996) A preliminary study on the microcommunity ecology of karst Cyanophyta in mat from Fendong Cave, Panxian, Guizhou. Carsalogica Sin 15:233–238
Tripathi SN, Chung IK, Lee JA (2007) Diversity and characteristics of terrestrial cyanobacteria near Gimhae City, Korea. J Plant Biol 50(1):50–59
Trono GC (1961) Studies of the Myxophyceae and Chlorophyceae of the Araneta University campus and vicinity. Araneta J Agric 8(3):139–192
Uher B (2010) Cyanobacterium *Petalonema alatum* BERK. ex KIRCHN. – species variability and diversity. Fottea 10:83–92
Viles HA (1988) Cyanobacteria and other biological influences on terrestrial limestone weathering on Aldabra: implications for landform development. Biol Sci Wash Bull 8:5–13
Vinogradova ON, Kovalenko OV, Wasser SP, Nevo E (1998) Species diversity gradient to darkness stress in blue-green algae/cyanobacteria: a microscale test in a prehistoric cave, Mount Carmel, Israel. Isr J Plant Sci 46:229–238
Wee YC, Lee KB (1980) Proliferation of algae on buildings in Singapore. Int Biodeterior Bull 16:113–117
Wetzel RG (2001) Limnology, 3rd edn. Academic, San Diego/London
Whitton BA (1971) Terrestrial and freshwater algae of Aldabra. Philos Trans R Soc Lond B 260:249–255
Whitton BA (2011) Cyanobacteria. In: John DM, Whitton BA, Brook AJ (eds) The freshwater algal flora of the British Isles, 2nd edn. Cambridge University Press, Cambridge, pp 131–158
Whitton BA, Diaz BM (1980) Chemistry and plants of streams and rivers with elevated zinc. Trace Subst Environ 14:457–463
Whitton BA, Gale NL, Wixson BG (1981) Chemistry and plant ecology of zinc-rich wastes dominated by blue-green algae. Hydrobiologia 83:331–341
Woodhead N, Tweed RD (1954) The freshwater algae of Anglesey and Caernarvonshire. North West Nat 25:392–435
Wujek DE, Cocuzza JM (1986) Morphology of hair of two- and three-toed sloths (Edentata: Brachypodidae). Rev Biol Trop 34:243–246

Wujek DE, Lincoln TA (1988) Ultrastructure and taxonomy of *Oscillatoria pilicol*a, a new species of blue-green alga from sloth hair. Brenesis 29:1–6

Wylie PA, Schlichting HE Jr (1974) A floristic survey of corticolous subaerial algae in North Carolina. J Elisha Mitch Sci Soc 89:179–183

Zackrisson O, DeLuca TH, Nilsson M-C, Sellstedt A, Berglund L (2004) Nitrogen fixation increases with successional age in boreal forests. Ecology 85:327–3334

Zackrisson O, DeLuca TH, Gentili F, Sellstedt A, Jäderlund A (2009) Nitrogen fixation in mixed *Hylocomium splendens* moss communities. Oecologia 160(2):309–319

Zimmermann U (1969) Ökologische und physiologische Untersuchungen an der planktischen Blaualga *Oscillatoria rubescens* D.C. unter besonderer Berücksichtigung von Licht und Temperatur. Schweiz Z Hydrol 31:1–58

Cyanobacterial Biofilms in Monuments and Caves

11

Patrizia Albertano†

Contents

Summary

Biofilm-forming cyanobacteria are widespread inhabitants of exposed stones in archaeological and historical sites and caves. Outdoors, these phototrophic biofilms are adapted to all types of stress imposed by growth at the air-rock interface and have developed the capacity to tolerate excess solar radiation, extreme temperatures and desiccation at different latitudes. Indoors, the typology of the cave or the characteristics of confined environments strongly select the microbial community according to light availability and air humidity. Interactions of cyanobacteria with rocky substrata serving as the source of mineral nutrients are based on the adhesion mechanisms and metabolic processes that allow the development of these biofilms. Both types of subaerial phototrophic community include cyanobacteria that support associated populations of heterotrophic populations of mostly very specialized species. The distribution of particular cyanobacterial taxa on monuments in urban or agricultural areas is related mostly to climatic conditions and the position and orientation of the hard surface with respect to water availability and air circulation.

The chapter provides an overview of the more recent studies on free-living subaerophytic cyanobacteria causing discolouration and erosion of lithic faces. Emphasis is on the biodeterioration of artworks due to physical and chemical processes caused by the growth of epilithic and endolithic organisms. The methods used for studying cyanobacterial communities on rocks and buildings of historic and artistic value are summarized, with the focus on conservation issues. Study techniques which are non-invasive of the underlying substrata are essential and it is important to identify the biodeteriogens responsible for the damage.

†deceased

P. Albertano
Dipartimento di Biologia, Universita degli Studi di Roma "Tor Vergata", Via della Ricerca Scientifica, 00133 Rome, Italy

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11.1 Cyanobacteria in Subaerial Phototrophic Biofilms

Cyanobacteria successfully colonize almost all illuminated environments, including some of the most hostile for life (Stal 2007). Rocks exposed to the atmosphere are inhabited by extremophilic cyanobacteria that play a basic role as primary producers in a variety of subaerial habitats such as cliffs and pinnacles of dry and humid regions (Pentecost and Whitton 2000; Chap. 10), hot and cold deserts (Wynn-Williams 2000; Vincent 2007) and caves (Hoffmann 2002), and might contribute to the future colonization of other planets (Grilli Caiola and Billi 2007; Billi 2012).

The colonization of rocks is due to lithophytic cyanobacteria growing as epiliths on surfaces and endoliths within the substrata, where they can reach a few millimetres below surface (Golubic et al. 1981). Their typically patchy distribution on solid substrata is due to local inhomogeneities of rock structure, such as differences in porosity, and other environmental differences in their microhabitats. Subaerial lithophytic cyanobacteria form phototrophic biofilms, surface-associated microbial communities with significant environmental and human impact. These multispecies consortia are built by photoautotrophic cyanoprokayotes (with associated microalgae) and chemoorganotrophs (heterotrophic bacteria and fungi) spatially organised in complex assemblages from different functional groups. Subaerial phototrophic biofilms are embedded in exopolymeric matrices, which mediate adhesion of the microbial communities to the underlying substrata (Karsten et al. 2007; Chap. 18). Because cyanobacteria can both photosynthesize and in many cases fix atmospheric nitrogen, they are primary colonizers of rocks and important lithobiont components of stone biofilms and can also enter into symbiosis with fungi and other phototrophs (Chap. 23).

The morphology, chemistry, physiology and ecology of naturally occurring phototrophic biofilms are as diverse as their constituent microorganisms. While the structure, growth dynamics and physiology of heterotrophic biofilms have been studied extensively, phototrophic biofilms have until recently received less attention, the best described being cyanobacterial mats (Stal 2000; Chap. 4). Similarly to aquatic microbial communities, cyanobacteria in subaerial biofilms generate energy and reduce carbon dioxide and atmospheric nitrogen, providing organic matter and oxygen to heterotrophs. This photosynthetic activity fuels processes and conversions in the biofilm community as a whole, including the degradation of organic compounds and the release of inorganic carbon, nitrogen and phosphorus required by the phototrophic fraction.

Variously coloured, blue-green, grey, brown, violet or black strips on natural rocks are caused by the growth of cyanobacteria and have long been known as "Tintenstriche" (Jaag 1945; Chap. 8). Epilithic biofilm-forming cyanobacteria discolour natural rocks, buildings and monuments on which they grow not only because of their chlorophyll, phycobiliproteins and carotenoids, but in many cases also UV-screening compounds, such as the yellow-brown scytonemins and the red to blue gloeocapsins (Chap. 19). The latter pigments, which aid survival under extreme solar exposure, can enhance markedly the visibility of cyanobacterial biofilms on rock surfaces. In contrast, endolithic cyanobacteria experience diminished solar radiation, because they occupy cracks and fissures or defined layers within the rock that reduce incident light down to as little as 0.001% surface irradiation (Friedmann and Ocampo-Friedmann 1984; Büdel 1999; Chap. 10).

An epilithic biofilm typically consists of a consortium of microorganisms resistant to variable stresses (poikilotolerant) from diverse taxa, including bacteria, cyanobacteria, algae, mosses, ferns, fungi and lichens. Lithobionts exposed to the atmosphere experience much harsher and more variable conditions than those in soil or water. The poorly buffered subaerial environment results in communities restricted in diversity and biomass (Gorbushina and Broughton 2009).

The metabolic activity of subaerial biofilms is sustained by their ability to retain water through the secretion of compounds that protect the cells from fluctuating light and water conditions and chelate nutrients for the growth of the enclosed microbial community. Generally, biofilms are composed by microbial cells, extracellular polymeric substances or secretions (EPS), biogenic and inorganic particles, and multivalent cations. Microbial strategies for adhesion to stone surfaces are based on the production and secretion at the cell surface of mucilaginous compounds. EPS is secreted by the microorganisms as glycocalyx, sheath or envelope, and acts as an adhesive that allows cells to stick to the substratum, and to form multispecies biofilms. Cyanobacterial capsules and sheaths are structured investments that form an integral component of the cell, whereas EPS loosely attached to the cells or exuded freely in the environment is termed mucilage (or slime) (Stal 2010).

Exposure to high solar radiation and substantial fluctuations in the availability of water restrict survival and growth of subaerial lithobionts. In arid environments, dew, fog, and sparse rain are the only sources of humidity. In extreme hot and cold deserts, dew condensation or melting snow provide organisms with sufficient water, whereas rock-inhabiting biofilms of temperate regions benefit from a temporary or regular supply by rainwater (Büdel et al. 2008, 2009). Even then, extreme temperatures accelerate water loss through evaporation or freezing (Wynn-Williams 2000). The scarcity of water in subaerial environments also imposes salt stress, and the production of osmolites by cyanobacteria is used as a mitigation strategy to protect proteins and pre-

vent desiccation-induced damage in combination with sheath pigments and cytoplasmic mycosporines (Billi and Potts 2000, 2002). High concentrations of sugar alcohols can accumulate inside cells to avoid excess water loss under drought conditions, providing compatible solutes and rapidly available respiratory substrates (Karsten et al. 2007). In the cryptoendolithic desert *Chroococcidiopsis*, the disaccharides trehalose and sucrose-6-phosphate have been shown to accumulate in response to osmotic stress, but data are lacking as to whether or not desiccation has a similar effect (Grilli Caiola and Billi 2007). *Chroococcidiopsis* and *Nostoc* species can dry without dying, a phenomenon known as anhydrobiosis, that allow cyanobacteria to escape the harsh outside climate inside porous rocks where they survive in a dry, metabolic state for prolonged periods. Even though the strategies underlying the ability of anhydrobiotic cyanobacteria to cope with prolonged desiccation are not fully understood, it seems probable that there is an interplay between protection and repair mechanisms (Billi 2009, 2011).

The morphology and diversity of subaerial cyanobacteria adapted to the lithobiontic life includes both unicellular *Synechococcus* only a few microns in diameter and filamentous forms with trichomes more than 30 μm wide. Over a hundred species have been recorded that belong to all subsections and orders of cyanobacteria. Hoffmann (1989) reviewed much of the information available then about lithobiontic algae in subaerial habitats using the terminology for microenvironments proposed by Golubic et al. (1981): epilithic, chasmoendolitic, cryptoendolithic and euendolithic. However, the diversity of lithobionthic cyanobacteria in subaerial biofilms is relatively little understood, and most taxa described so far are still considered to be cosmopolitan, with only a few rare or endemic species (de los Rios et al. 2007; Uzunov et al. 2007).

The identification of cyanobacteria associated with surfaces is usually difficult, because most taxa exhibit few morphological features. However, their taxonomy and systematics is progressing as a result of the sequencing of 16S rRNA and other genes in combination with the analysis of cytomorphological characters (Wilmotte 1994; Wilmotte and Herdmann 2001). The taxonomy of over 150 genera and 1,500 species listed under the International Code of Botanical Nomenclature has been subject to various revisions aimed to define genera as more homogeneous taxonomic groups mainly on the basis of ecological, morphological, and ultrastructural characters (Komárek and Anagnostidis 1998, 2005; Komárek 2010; Komárek and Hauer 2010). Currently the orders of the phylum Cyanobacteria/Cyanophyta correspond to the five subsections accepted by the International Code for Nomenclature of Bacteria mostly on the basis of biochemical and genetic features. Therefore, most researchers are now applying a polyphasic approach that combines phenotypic and genetic studies grouping sequences with more than 95% into the same genus and 97.5% similarity into operational taxonomic units (OTUs) or phylotypes that may correspond to one (or more) species clearly distinct from others (Rajaniemi et al. 2005; Foster et al. 2008; Zakhis et al. 2008). The use of this approach is providing new insights on the species richness of subaerial communities, where there are many records for new cyanobacterial species and phylogenetic results support the establishment of new genera (Rindi 2007; Bruno et al. 2009).

Marked variations in environmental parameters can lead to time-dependent dynamics of subaerial microbial communities and to the establishment of different micro-habitats at different times. Early studies on the influence of humidity showed the presence of a characteristic epilithic cyanobacterial association on exposed limestone rocks of the Dinaric-Alps (*Scytonema-Gloeocapsetum*) that could be replaced by a different one in shaded areas (*Aphanocapsa-Chroococcetum*), while increased humidity supported the growth of various assemblages at high (*Tolypothricetum byssoideae*, *Dichothricetum gypsophilae*, *Calothricetum parietinae*) or low light conditions (*Schizotricetum heufleri, Schizothricetum lardaceae, Hydrocoleetum homoeothrichii*) (Golubic 1967). Phylogeny and biogeography of rock-inhabiting microbes in subaerial communities is showing their similarity on different continents and the cosmopolitan distribution of some rock settlers (Gorbushina and Broughton 2009). This may also apply to epilithic and endolithic cyanobacteria inhabiting similar habitats in other geographical areas (Sigler et al. 2003; Taton et al. 2006).

11.2 The Monument Environment

During the first half of the nineteenth century there were already reports on biological growths on rocks and monuments, such as by A.V. Humboltd, Charles Darwin and J.C. Ehrenberg (Adhikary 2000b). Subsequent accounts of subaerial lithophytic cyanobacteria documented their wide occurrence throughout the world during the past century. Subaerial cyanobacterial biofilms commonly develop on or within man-made surfaces as on any solid mineral substratum exposed to the atmosphere, and are ubiquitous at all latitudes on monuments and buildings, but particularly abundant in warm temperate and tropical regions (Ortega-Calvo et al. 1993; Tripathy et al. 1997, 1999; Gaylarde and Gaylarde 2000; Ortega-Morales et al. 2000; Tomaselli et al. 2000; Pattanaik and Adhikary 2002a; Crispim et al. 2003; Crispim and Gaylarde 2005).

In cultural heritage studies, epilithic cyanobacterial assemblages are usually described as patinas, stone alterations and crusts when they are obvious to the naked eye (Figs. 11.1, 11.2, and 11.3) (Urzì et al. 1992). As mentioned above, the discolouration is due to cyanobacterial and eukaryotic algal pigments. The extracellular pigments may not only protect

Fig. 11.1 Phototrophic biofilms on outdoor monuments in the temperate climate of Rome, Italy: (**a**) Colonization of organic nutrient enriched rock surfaces and porous mortar layers of a Roman wall on the posterior of the Pantheon temple; (**b**) Black strips on a marble statue and (**c**) spots on one column at the *Terme di Diocleziano* in a heavily air polluted area of the city. Note the growth of cyanobacteria on the wall in the shaded area behind the column, where humidity is high

against high visible and UV irradiance (Roy et al. 1997; Adhikary 2004), but perhaps also help protect against desiccation and temperature stresses (Fleming and Castenholz 2007). The settlement and persistence of multispecies biofilms on exposed rocks relies on their ability to face high solar radiation, repeated cycles of desiccation and rewetting, prolonged desiccation, temperature fluctuations and nutrient limitations (Gorbushina 2007).

The diversity and the deteriogenic activity of epilithic and endolithic cyanobacterial communities depend on the availability of light, water, carbon and other nutrients to sustain microbial metabolism that in turn causes the irreversible transformation and biomineralisation of substrata. Endolithis may be growing in cracks and pores and boring into rocks, although this might be obscured by superficial algal growths, and consequently overlooked (Pentecost 1992). Most of the

Fig. 11.2 Discolouration patterns on surfaces: (**a**) The Bernini's Elephant statue in the Rome city center (Italy) (**b**) Façade of St Boniface church in Brussels with black sulphated crusts and photrotrophic biofilms in more humid areas. Differently coloured strips are present on the most exposed and dry upper part of the façade; (**c**) *Terme di Diocleziano* and (**d**) Trevi's fountain in Rome (Italy) with subaerial growth of cyanobacteria on marble in sun sheltered areas and constant wetness

relatively few reports on endolithic cyanobacteria on buildings describe the presence of coccoid cyanobacteria (Saiz-Jimenez et al. 1990; Ortega-Morales et al. 2005). Diverse bacterial communities in limestone of Maya archeological sites have shown that the endolithic community is distinctly different from the communities on limestone surfaces due to the influence of the physical and chemical properties of the calcareous stone materials (McNamara et al. 2006). It is, however, not yet clear to what extent taxonomic diversity influences the weathering, nor the quantitative differences between damage caused by endoliths compared to that by epiliths at the same site.

Large seasonal variation of UV radiation and low maximum values are characteristics of high latitudes, while low latitudes impose stronger exposure that elicites cell responses to survive the damaging effects of UV radiation similar to high altitude exposed sites (Castenholz and Garcia-Pichel 2000). In monuments, stone surfaces at different heights usually harbour different cyanobacterial communities, with the less desiccation-tolerant species developing close to the ground where humidity and nutrients are high, while poikilotolerant taxa colonize high and more exposed levels.

The most important limiting factor for the growth of phototrophs is light, the intensity and quality of which depend on the location and specific architectural features of the monument. The average intensity of light reaching a surface on a sunny day ranges from 1,000 to 2,000 μmol photon m^{-2} s^{-1} at mid-day. Light is the driving energy that sustains phototroph

Fig. 11.3 Discolouration of Hindu temples in Bhubaneswar, Orissa, India: (**a**) The complex of Lingaraj temples and (**b**) close-up of sandstone with carvings completely covered by dry cyanobacterial biofilms; (**c**) Exterior of one temple at the Khandagiri cave colonized by black biofilm strips on rock

development, and appropriate irradiance values or prolonged lighting periods support extensive growth of photosynthetic microorganisms. However, light absorption and scattering at the surface can differ markedly depending on the type of rock. The presence of airborne particles and biofilm of various thickness readily attenuate light within the community and most cyanobacteria, that are usually adapted to low photosynthetic photon flux densities, are often photoinhibited. When available wavelengths vary, cyanobacteria can adjust their light harvesting pigments to optimize light absorption. These photobiological features are at the basis of the acclimation processes of phototrophic biofilms and are reflected by their photosynthetic performance and hence growth.

Subaerial microbial communities are hold together and to the substratum by the EPS produced by the phototrophic and heterotrophic component. As a result characteristic microenvironments are originated, which allow the survival of EPS-embedded microorganisms in harsh environments (Stal 2000). EPSs are polyelectrolytes of high molecular weight made by polysaccharides (up to 90%), proteins and nucleic acids, that contribute to trapping water. These compounds, present as gels in capsules and sheaths or solubilised into the mucilaginous biofilm matrix, are synthesized by one or more of the microbial groups present within the biofilm and contribute to the initial stages of biofilm formation and to the subsequent co-aggregation and stabilisation of a multispecies community (Rickard et al. 2003). EPS also actively participate in the weathering process by contributing to the binding of solubilized minerals (De Philippis and Vincenzini 1998; Pereira et al. 2009) and several studies have reported on the high contents of chelating uronic acids and sulphated groups of the heteropolysaccharides produced by cyanobacteria on lithic faces of archaeological sites (Albertano and Bellezza 2001; Bellezza and Albertano 2003; Bellezza et al. 2005).

Colonization and growth of cyanobacteria and associated microorganisms accelerates weathering and soiling of rocks (Warscheid and Braams 2000; Gaylarde and Morton 2003). Phototrophic and heterotrophic microorganisms cause biodecay and biotransformation of rock substrata while carrying

out normal metabolic activity (Urzì 2004; Gorbushina 2007). Bioerosion of archaeological and artistic lithic surfaces by microbial communities colonizing monument surfaces has been observed in many geographical areas and there has been an increasing concern about the possible damage to the cultural heritage caused by microbial activities. This has stimulated a considerable number of studies in more recent years (Ortega-Calvo et al. 1995; Ciferri 1999; Crispim and Gaylarde 2005; Ortega-Morales 2006). Nevertheless, the deterioration mechanisms of the large range of rock types with different mineralogical characteristics and the varying weathering responses under different climatic and environmental conditions are still far from fully understood. Like all materials, rock is subject to inexorable deterioration, especially if exposed to the weather. Air pollution is one of the major factors responsible for depositing chemical substances and biological agents on outdoor stone surfaces (Zanardini et al. 2000; Nuhoglu et al. 2006).

Pigment formation can have undesirable masking effects on the undelaying work of art. The chemical interactions of pigments with constituents of the substratum, trace metals and pollutants can further alter the mineral composition of the stone. Variously pigmented patinas with patchy distribution due to non-homogeneous growth appear on lithic faces or fissures and cracks or beneath the surface, depending on the conservation state of artefacts. The various microhabitats within a single monument can be colonized by different phototrophs, because of environmental constraints on the phenotypic expression of forms adapted to particular ecological niches.

Another important, but largely neglected, aspect of the ecology of monuments is the impact on humans of active biomolecules produced by cyanobacteria and associated microorganisms. Exposure to toxins is possible by inhalation of the aerosolized cells and molecules. The few reports about this deal with the risks to human health of short- and long-term inhalation effect of toxic volatile compounds produced by biodeteriogenic actinobacteria (Salkinoja-Salonen et al. 2003) and toxigenic airborne cyanobacteria (Kumar et al. 2007).

The development of phototrophs results in physical and chemical damage due to time variable external loadings in conjunction with environmental moisture, heat, freeze-thaw action, shrinkage, chemical and biological dissolution and corrosion. A recent overview of issues related to biodeterioration and conservation of cultural properties around the world provided insights on the interactions between stone and microorganisms, the way they can enhance or retard the overall rate of degradation, and outlined the predominance of bacterial endoliths in calcareous and siliceous stone monuments (Scheerer et al. 2009). Other authors have discussed the threats to a wide range of heritage materials and monuments by biological and chemical agents of decay and brought together contributions from the field of plant biology related to the biodeterioration, emphasising correlations between deterioration processes, organisms and environment, and describing case studies in various environmental and climatic conditions and diverse geographic settings (May et al. 2008; Caneva et al. 2009).

11.3 Outdoor Monuments

11.3.1 Overview

Microbial colonisation depends on liquid water, that is only periodically available in the form of rain, dew, or condensation of atmospheric humidity. Therefore, the EPS is of great importance for epilithic and endolithic cyanobacteria, as it retains water and acts as osmoprotectant, nutrient reservoir and toxicant chelator. Although atmospheric pollution is generally recognised as a significant physico-chemical factor in the deterioration of cultural properties, biodeterioration caused by cyanobacteria has rarely been analysed in relation to air pollutants. In the presence of atmospheric pollution and a humid environment, calcareous rock is transformed into hydrated calcium sulphate (gypsum, $CaSO_4 \cdot 2H_2O$). This should be removed from surfaces, as hygrometric fluctuations lead to dissolving and crystalizing processes and subsequent mechanical stress. In areas sheltered from the rain, gypsum embeds mineral and smog particles, leading to the formation of the so-called black crusts that represent chemical alteration and aesthetic disfigurement (McNamara and Mitchell 2005). Ortega-Calvo et al. (1994), studying the effect of black crusts on *Gloeothece* sp., showed that when gypsum was removed from the rock surfaces of Seville cathedral and added to a mineral salt medium, the surface dissolved slowly, releasing sulphate that was progressively incorporated into the cyanobacterial sheath and used for its growth.

Sedimentary rocks have been extensively used in the construction of monuments and historical buildings. Since the first reports in the 1960s on biodeterioration of stone artworks by phototrophic organisms (Raistrick and Gilbert 1963; Lefèvre et al. 1964), a large number of studies has already assessed the occurrence of cyanobacteria and algae on sedimentary stone materials and cave walls from cultural heritage. However, the effect of weathering by subaerial cyanobacterial communities has only seldom been quantified in nature, though the few calculations made on limestone reported rates between 0.5 and 3 mm/100 years along with exfoliation and pitting of stone surfaces (Pentecost and Whitton 2000).

Cyanobacterial biofilms have been found on limestone and sandstone man-made surfaces of very different types and mineral composition. Floristic records have been reported

for the sandstone of Hindu temples and monuments in various part of India (Pattanaik and Adhikary 2002a, b) and Angkor temples in Cambodia (Lan et al. 2010), marbles in Italy (Tomaselli et al. 2000), granite in Serbia (Grbič et al. 2010); terracotta at Bishnupur temples in West Bengal and brick walls in Tamil Nadu, India (Pattanaik and Adhikary 2002b). Several authors summarized the extant knowledge about taxa recorded for different, mostly limestone, mineral substrata, providing relatively detailed lists (Ortega-Calvo et al. 1991; Adhikary 2000b; Kovácik 2000; Uher et al. 2005; Macedo et al. 2009).

The cyanobacterial component of organisms visible in biofilms over a unit area has been reported to range roughly from 17% to 35% for European versus 25–66% for Latin-American monuments (Gaylarde and Gaylarde 2005). However, quantitative data on biomass for monuments are scarce, making it difficult to compare with natural communities in other subaerial environments; the diversity of methods for expressing the value per unit area adds to the problem.

The development of biofilms on lithic faces is closely related to the environmental humidity necessary for microbial growth. Porosity and hygroscopicity of materials, capillary water absorption and relative humidity strongly influence water availability for the phototrophs, so calcareous substrata are colonized more easily than granite. A survey of the literature on biodeterioration, followed by experimental studies using microscopy and genetic methods (ARDRA), led Tomaselli et al. (2000) to recognize different species associations from different types of environment: *Chroococcus minor, Myxosarcina concinna*, *Gloeocapsa biformis*, *Pleurocapsa* and *Scytonema* species with calcareous lithotypes; *Phormidium tenue*, *P. autumnale* and *Microcoleus vaginatus* with siliceous rocks; *Nostoc puntiforme*, *N. muscorum*, *Chroococcidiopsis* and *Leptolyngbya* species with frescoes and plasters. *Gloeocapsa alpina* isolated from a Portuguise church and inoculated on limestone, granite and white marble to test the bioreceptivity of the different materials, grew on carbonate, but not silicate, substrates (Miller et al. 2006). Sometimes a particular organisms is especially important in a particular region, such as *Gloeocapsa novacekii* on sandstone monuments in Belgrade (Serbia) (Grbič et al. 2010). Other studies have emphasized the importance of factors other than the chemical composition of the substrate. Barberousse et al. (2006a), using multivariate analysis, concluded that precipitation, hygrometry, thermal amplitude, distance from the sea and proximity to vegetation were the major factors influencing the distribution of phototrophic biofilms on building facades in France. An assessment of data for monuments in European Mediterranean countries (45 case studies, 1976–2009) concluded that the occurrence of cyanobacteria on the main lithotypes (marble, limestone, travertine, dolomite, granite, sandstone, terracotta) was only secondarily related to the lithotype, the main factors being porosity, roughness and permeability of the surface (Macedo et al. 2009). Overall, the majority of literature does not indicate that cyanobacterial populations on exposed surfaces of outdoor monuments are distinctive according to mineral type, but this must be viewed with caution, because so many studies are based on insufficiently critical taxonomic identification. The need for this to be more accurate is made clear by Kaštovsky et al. (2010) in their revised checklist of cyanobacteria for the Czech Republic (392 species). In an assessment of common to rare aerophytic epilithic forms on four types of rock environment (wet walls, granite and sandstone, calcareous, serpertine and ultrabasic), taxon specificity was evident for surfaces with higher water availability versus dry ones, and for acidic versus basic ones, although *Aphanothece caldariorum* proved an exception (Table 11.1). Most of the taxa recorded on outdoor monuments have also been reported from natural environments, so literature about the latter can provide further helpful information e.g. Uzunov et al. (2007).

11.3.2 Temperate and Mediterranean Climates

At latitudes above the tropics, most accounts for cyanobacteria on outdoor monuments have come from Europe. These include biofilms on marble statues and fountains in Tuscany (Tomaselli et al. 2000; Cuzman et al. 2010), archaeological sites, churches and historic buildings in France, Greece, Italy, Serbia, Spain, Ukraine, Turkey, Belgium, Germany, Ireland and Sweden (Ortega-Calvo et al. 1993; Adhikary 2000b; Darienko and Hoffmann 2003; Kovácik 2000; Macedo et al. 2009; Grbič et al. 2010). Tomaselli et al. (2000) made a literature survey to quantify the relative importance of taxa. *Phormidium* was the most widespread genus (seven species, *P. autumnale* and *P. tenue* the most frequent), with the next most important being *Nostoc* (six species, mostly *N. punctiforme*), *Microcoleus* (four species, mostly *M. vaginatus*) and *Plectonema* (four species, mostly *P. boryanum*). Values for building facades in France (Barberousse et al. 2006b) gave *Cyanosarcina parthenonensis* (28%), *Chroococcus lithophilus* (21%), *Gloeocapsa sanguinea* (15%) for coccoid species, and *Calothrix pulvinata* (21%), *Leptolyngbya foveolarum* (18%) and *Phormidium corium* (13%) for filamentous species. The unsightly effect of dark-coloured stains in Renaissance and Baroque cloisters of the Santo Domingo College (Orihuela, Alicante, S-E Spain), was due to 14 epilithic species with Chroococcales and Oscillatoriales the most important (Sánchez-Antón and Asencio-Martínez 2007). *Chroococcus* and *Tolypothrix* showed the highest species diversity, while *Pseudocapsa* was the most frequent genus. Of the 37 genera and 96 species listed by Macedo et al. (2009), the most widespread taxa on all substrata

Table 11.1 Valid names of common and rare aerophytic cyanobacterial species reported as epiliths (or chasmo-endoliths*) on rocky substrata in the Czech Republic (Data adapted from Kaštovsky et al. 2010)

Wet walls	Granite and sandstone	Calcareous rocks	Serpentine and ultrabasic rocks
Aphanocapsa muscicola	*Aphanothece caldariorum*	*Aphanocapsa parietina*	*Aphanothece caldariorum*
Aphanothece bullosa	*A. saxicola*	*Aphanothece castagnei*	*Chroococcus spelaeus*
Chroococcus cohaerens	*Chr. turgidus*	*Calothrix parietina*	*Entophysalis atroviolacea*
Chr. helveticus	*Chr. various*	*Chlorogloea novacekeii*	*Gloeocapsa alpina*
Cyanosarcina huebeliorium	*Cyanothece aeruginosa*	*Chl. microcystoides*	*G. compacta*
Gloeocapsa punctata	*Dichothrix orsiniana*	*Chondrocystis dermochroa*	*G. novacekii*
G. rupicola	*Gloeocapsa bituminosa*	*Chroococcus ercegovicii*	*Gloeocapsopsis chroococcoides*
Gloeothece palea	*G. fuscolutea*	*Chr. pallidus*	*Gl. dvorakii*
Glo. tepidariorum	*G. haematodes*	*Chr. spelaeus*	*Gl. pleurocapsoides*
Leptolyngbya angustissima	*G. kuetzingiana*	*Chr. turicensis*	*Hassallia byssoydea*
L. carnea	*G. reicheltii*	*Chr. various*	*Scytonema crustaceum*
L. cataractarum	*G. sanguinea*	*Dichothrix gypsophila*	*Stigonema panniforme*
L. cebennensis	*Homoeothrix janthina*	*Entophysalis atroviolacea*	*St. tomentosum*
L. compacta	*Microchaete brunescens*	*Gloeocapsa aeruginosa*	
L. edaphica	*Scytonema mirabile*	*G. alpina*	
L. foveolarum	*Stigonema hormoides*	*G. biformis*	
L. hennigsii	*St. informe*	*G. compacta*	
L. subtilissima	*St. minutum*	*G. nigrescens*	
Merismopedia minima		*G. novacekii*	
Microcoleus subtorulosus		*G. rupestris*	
M. vaginatus		*G. violacea*	
Nostoc calcicola		*Gloeocapsopsis chroococcoides*	
N. microscopicum		*Gl. dvorakii*	
N. punctiforme		*Gl. pleurocapsoides*	
Oscillatoria rupicola		*Gloeothece confluens*	
Phormidium kolkwitzii		*Glo. rupestris*	
P. papyraceum		*Hassallia byssoydea*	
*P. rimosum**		*Leptolyngbya gracillima*	
P. schroeteri		*Phormidium rimosum**	
P. violaceum		*Rivularia haematites*	
Pseudophormidium tenue		*Scytonema crustaceum*	
Scytonema hofmanni		*S. drilosiphon*	
S. myochrous		*Tolypothrix bouteillei*	
Symploca muralis		*T. elenkinii*	
Sy. muscorum			

were *Chroococcus, Gloeocapsa* and *Phormidium,* with *Chroococcus minor*, *Gloeocapsa biformis* and *Phormidium foveolarum* the most frequent species. *Pleurocapsa minor* on marble associated with fountains was also one of the most frequently recorded taxa, whereas other forms of *Pleurocapsa* and also *Scytonema* on Mediterranean monuments remain unidentified to the species level.

Fountains and nymphaea are characterized by having some areas continuously or sporadically wetted by running water, while others are always dry. The presence or absence of water, its physical and chemical features and the exposure to direct or shaded sunlight all influence phototroph distribution (Pietrini and Ricci 2009). A recent overview on the biological patinas that developed in fountains in Italy and Spain compared the biodiversity of the different biotopes in relationships to water availability, light exposure, and lithotypes (Cuzman et al. 2010). Using microscopy combined with automated ribosomal RNA intergenic spacer analysis and the principal component analysis of obtained profiles, the authors identified 32 cyanobacteria showing a precise match of molecular phylogeny data and morphological identification of cyanobacterial isolates, that separated in four clusters according to the microenvironmental conditions of each fountain. Species of *Chlorogloea*, *Chroococcus*, *Pleurocapsa* and *Phormidium* were the most common colonizers of wet spots along with *Calothrix* and *Nostoc*, whilst *Chroococcidiopsis* occurred in dry areas (Cuzman et al. 2010).

11.3.3 Tropical Climates

Kumar and Kumar (1999) provided an overview of general biodeterioration problems in tropical climates and mentioned cyanobacteria as one of the main biodeteriogenic groups of organisms. In the case of Latin America, most surveys of cyanobacteria in biofilms on historical buildings have been made at archaeological sites on Mexico (Gaylarde et al. 2001; Ortega-Morales et al. 2000; McNamara et al. 2006) and Brazil, though some have also been investigated in Argentina, Bolivia and Peru (Gaylarde and Gaylarde 2000). Ortega-Morales (2006) concluded from the literature that Pleurocapsales were the main colonizers at the Mayan site of Uxmal in Mexico, but *Synechocystis*, *Gloeocapsa* and *Xenococcus* were generally the dominants elsewhere on monuments in Latin America, accompanied by *Lyngbya*, *Plectonema* and *Nostoc.* Ramírez et al. (2010), who studied buildings at Palenque, another archaeological site in Mexico, described the three-dimensional structure and distribution on rock, stucco and concrete of photosynthetic microorganisms in the biofilms dominated by the desiccation-tolerant *Scytonema guyanense* and *Asterocapsa divina,* that occupy respectively dry and more shaded-humid habitats. Cyanobacterial diversity was higher at biofilm surfaces under low sunlight and prolonged wet conditions than those exposed to full solar radiation. This led them to discuss the implications for the development and persistence of phototrophic species able to withstand temporal heterogeneity resulting mainly from the alternating wet and dry seasons. The more vulnerable species seemed to grow in the bottom layer of the biofilm, their persistence depending on the ability to tolerate the annual desiccation period, whereas species on top probably took advantage of fast growth rates under favourable rainy conditions.

Reports on cyanobacteria on Asian monuments include a few for Cambodia (Lan et al. 2010), Korea (Fusey and Hyvert 1964; Tripathi et al. 2007), Iran (Mohammadi and Krumbein 2008), and most extensively in India (Roy et al. 1997; Tripathy et al. 1997; Adhikary 2000a, b; Pattanaik and Adhikary 2002b). A total of 30 species belonging to 13 different genera were reported by Tripathy et al. (1999); their detailed account described seven species of *Tolypothrix* along with *Gloeocapsopsis dvorakii*, *Lyngbya corticola, Phormidium truncicola* and *Plectonema puteale* as the major components of crusts and tufts on Indian stones. Species of *Gloeothece*, *Chroococcidiopsis*, *Myxosarcina*, *Plectonema*, *Calothrix*, *Nostoc*, *Chlorogloeopsis*, *Fischerella* and *Hapalosiphon* appeared in the enrichment culture as minor components of the biofilms. A study by Pattainak and Adhikary (2002) identified 46 cyanobacterial taxa at Indian archaeological sites and caves in Orissa, Maharashstra, Karnakata, Uttar Pradesh, Tamil Nadu, Delhi and West Bengal. These phototrophic communities showed a marked capacity to tolerate the extreme environmental conditions thanks to the stability of their chlorophyll *a*, ability to grow in the dark, production of heat-shock proteins, scytonemin and mycosporin-like aminoacids (Roy et al. 1997; Adhikary 2000a, b). In a study by Pattanaik et al. (2004) the scytonemin was sufficient to cause a dark pigmentation of the stone, while heat-shock proteins supported resistance to temperatures >60°C.

More recently, some of the thousand sandstone temples in the Golden triangle of Orissa, N-E. India, which date back to the eighth to twelfth centuries (AD), have been investigated to understand the processes which have led to changes in stone microhabitats since the 1990s. These have been caused largely by a combination of unsuitable conservation strategies and the effects of climate change, which together have led to the modification of the microbial communities described above and increased corrosion of the sandstone. The following are some of the studies relating to cyanobacteria.

These architectural complexes have an extensive cover formed by two main types of cyanobacterial biofilm. This is a stable black or brownish crust during the dry season (Fig. 11.3) and a thick blue-green biofilm during the monsoon period. The latter biofilm survives the extended period of drought, temperature and high radiation stress in a vegetative state and metabolic activity revives soon after rewetting (Adhikary 2004). Among the 57 taxa reported for building facades in India only *Scytonema pseudoguyanense* and *Gloeocapsa kuetzingigiana* thrive on sandstone during the period of extreme temperature and desiccation (Lakshmi and Adhikary 2008). However, variations were detected in the type of coccoid and filamentous species during a 3-year study, with an increase of the total number of phototrophic taxa particularly during the monsoon season. The most extensive alterations of the lithic faces were still due to biofilms of *Tolypothrix* and *Lyngbya* during the dry season (Fig. 11.4), while samples collected during the rainy season showed an almost stable number of cyanobacterial taxa, but also a few eukaryotic algae (S.P. Adhikary, personal communication).

Tolypothrix byssoidea (Adhikary and Satapathy 1996) is one of the most common cyanobacteria on exposed surfaces of Hindu temples thanks to its scytonemin and mycosporin-like amino-acids absorbing UV radiation, together with a range of heat-shock proteins (Adhikary 2000a, b; Pattanaik et al. 2007). The analysis of biofilm matrices, cyanobacterial and bacterial capsules revealed the abundance of compounds acting in the protection from water limitation and dangerous radiation. The heteropolysaccharides extracted from some isolates were shown to have a high affinity for bivalent metal cations, i.e. calcium, magnesium and iron, suggesting their ability to actively contribute to weakening the mineral substrata. Eleven to 12 neutral and acidic sugars were detected in the slime secreted by these strains, two of which showed 98% sequence similarity of the 16S rRNA gene to a strain of *Chroogloeocystis siderophila* (Rossi et al. 2012).

Fig. 11.4 Cyanobacterial biofilms on Hindu temples: (**a**, **b**) Abundant development of biofilms on the walls during the monsoon period leads to the formation of black crusts during the dry season; (**c**) Severe exfoliation and erosion of sandstone caused by the detachment of biofilms; (**d**) Dried cyanobacterial crusts in a sheltered area

Büdel et al. (2004) reported a different weathering mechanism in South African sandstone formations, where the cryptoendolithic *Choococidiopsis* sp., *Nostochopsis lobata* and *Trichocoleus* cf. *sociatus* induced weathering by substrate alkalization due to positive net photosynthesis coupled to a pH increase to 9.5–10.5, values high enough to solubilize silica. As a result of deprotonation of Si-O-H bonds, the upper rock part was loosened and then eroded away by wind and water flow. Intermittent swelling of the EPS during water uptake also played a role in the final loosening of rock flakes. This special type of 'exfoliation' seems to be widely distributed in Africa and other continents and probably affects sandstone monuments around the world.

11.4 Caves and Other Subterranean Sites

11.4.1 The Cave Environment

Caves with rock art are widespread in several countries in all continents. Many cavities occur at various depths in a cave system due to the continual seepage and flow of water through the deposits, while underground rivers may eventually carve their way through a mountainside, creating openings and entrances to the outside. Caves are usually in connection with the outdoor environment, and thus subjected to climatic and microclimatic shifts. The most common caves are solutional caves, called limestone caves for the common type of soluble rock in which they form. Limestone caves are widespread all over the world and can range from a few metres to many kilometres in length and depth. Weak carbonic acid reacting with the chemicals in the rock, dissolves and erodes away the limestone as the water filtered into the underlying depths of sediments. Sandstone, also known as arenite, is a sedimentary rock of clastic origin composed mainly of sand-sized minerals or rock grains, mainly quartz and/or feldspar. Depending on the types of mineral component, some sandstones are resistant to weathering, while others are more friable. Sandstone caves are shallow caves that form at the base of cliffs, carved out by water and wind. The water loosens the natural cement holding the sand particles together, then the moving water, while wind carries away the sand grains.

Large hollow limestone cavities and smaller sandstone caves formed over thousands of years have become colonized by a

Fig. 11.5 Indoor development of phototrophic biofilms: (**a**) Roman frescoes at the Domus aurea and (**b**) wall paintings at the St. Domitilla catacombs of Rome (Italy) disfigured by the presence of thick biofilms; (**c**) Patchy distribution of cyanobacterial colonies on top of the wall and ceiling of a corridor and (**d**) on the vault of an "*arcosolium*" all occurring on plaster in close proximity to lamps

range of specialised organisms, including cyanobacteria. The natural cave environment is usually characterised by high relative humidity, although dry caves are also known, stable temperature throughout the year and light gradients provided by solar radiation at the entrances or penetrating through holes in the ceilings (Pentecost and Whitton 2000; Hoffmann 2002).

Caves have always been natural attractions for man and the remarkable mural paintings on the walls of Lascaux caves, in southern France (Lefèvre 1974), and the Altamira caves, in northern Spain, witness the presence of prehistoric men and their artistic ability. Numerous prehistoric caves, which have been naturally closed for thousands of years, have been discovered in the last two centuries, and then opened to the public with dramatic consequences on the cave microclimate (Gonzalez et al. 1999). Stones and mural paintings in caves and other hypogeal environments (crypts, tombs, etc.) frequently suffer from biodeterioration (Figs. 11.5 and 11.6), and the scarce literature on this topic is at last starting to increase, though slowly (Urzì et al. 2010). This includes not only cyanobacteria, but new chemoorganotrophic bacterial species.

In general, high values of humidity along with light conditions, and probably also nutrient input, appear to be the most important factors allowing growth of phototrophs here (Albertano et al. 2009; Pietrini et al. 2009). Caves are usually oligotrophic environments, where primary production depends on well-established autotrophic communities, both chemoautotrophic and photoautotrophic. However, cyanobacterial exudates and cell debris along with inputs of organic matter from above ground may support the growth of several chemoorganotrophic microbes.

The transport of viable cells to underground sites is due to air currents, water flow and contamination by animals and humans. Colonizing microorganisms are usually distributed on the mineral surface layer, but a few develop beneath it or actively bore into the mineral substrata. Chasmoendolithic cyanobacteria were observed in a cave in S-E. Spain (Asencio and Aboal 2000a), while monospecific assemblages formed by *Chlorogloea* sp. or mixed populations of *Chroococcus spelaeus* associated with *Aphanocapsa muscicola*, *Chroococcus turgidus*, *Gloeocapsa biformis* and *Leptolyngbya gracillima* were found inside a limestone cave in Greece (Lamprinou et al. 2009). Pleurocapsalean endoliths occur on the salted-ceiling of a limestone tunnel at the Edzna pyramid in Yucatan, Mexico (Ortega-Morales et al. 2005).

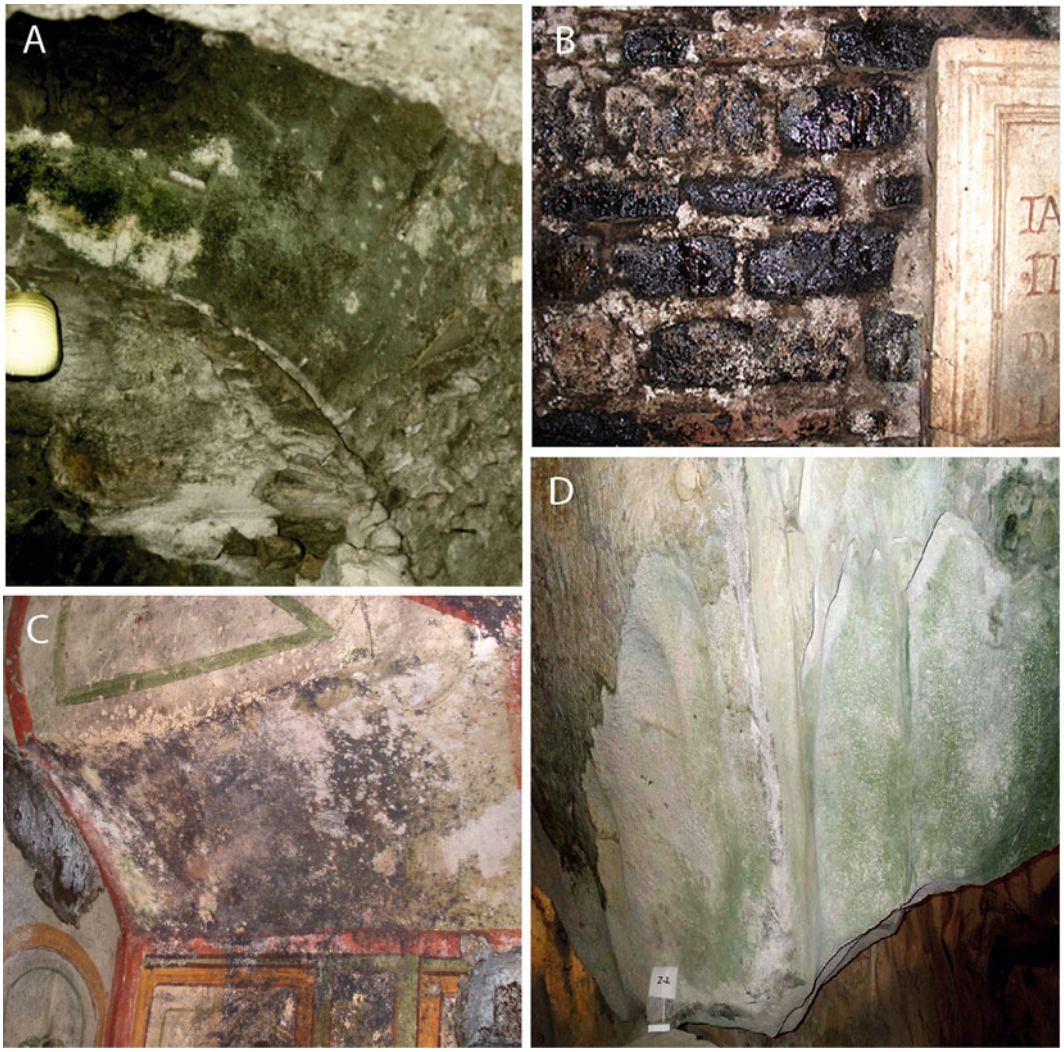

Fig. 11.6 Cyanobacterial biofilms on different lithotypes indoors: (**a**) Tufa rock at the Catacomb of Priscilla, Rome; (**b**) Brick wall in St. Domitilla catacombs, Rome; (**c**) Wall paintings inside the Ocean's *cubiculum* at St. Callistus catacombs, Rome; (**d**) Limestone at the Cave of Bats Zuheros, Cordoba, Spain

In general, phototrophic communities colonize surfaces at the entrances, but most can be found inside tourist caves, where light allows the growth of cyanobacteria, green algae, diatoms and lichens (Roldán et al. 2004, 2006). These associations known as 'lampenflora', develop in natural and artificial caves around lighting sources (Dobat 1998). Since humidity is usually high and temperature stable, the light energy drives microbial communities towards autotrophy that in turn supports associated heterotrophs. The photosynthetic communities that inhabit the caves are mainly epilithic on the surface of rocks speleothems, stalactites and stalagmites, that provide them with a variety of ecological niches (Roldán and Hernández-Mariné 2009).

Similarly to caves, phototrophic biofilms develop inside hypogeal archaeological sites on lithic faces near artificial light sources and in areas adjacent to entrances or openings to the surface, such as wells and air vents. These regions show a range of microclimatic conditions, reflecting the transition from the outdoor environment to the influence of the constant humidity, temperature and poor air circulation of the inner regions. Much also depends on the size and architecture of the hypogea. In the outdoor-indoor transitional areas, phototrophic communities include saxicolous lichens, which are absent in the deepest parts (Roldán and Hernandez-Mariné 2009). Several studies have reported or reviewed the presence of abundant populations of cyanobacteria in caves of Australia, Belgium, China, Croatia, France, Israel, Italy Hungary, Slovenia, Spain, UK and USA (Abdelahad and Bazzichelli 1988; Abdelahad 1989; Hoffmann 1989; Garbacki et al. 1999; Pentecost and Whitton 2000; Smith and Olson 2007; Mulec et al. 2008). Although few studies have attempted to quantify biomas, Mulec et al. (2008) showed that the maximum concentration of chlorophyll a per unit area of lampenflora biofilms in Slovenian karst caves was slightly

Table 11.2 Some recorded ranges (min and max values) of the environmental conditions that supported the development of lithobiontic subaerial populations of cyanobacteria in caves and other archaeological underground sites of the Mediterranean area with different stone substrata

Location and substratum	PPFD (μmol m^{-2} s^{-1})	Temperature (°C)	Air humidity (RH%)	References
Leontari cave, Attica, Greece – L	0.0001–0.64	11–17.5	77–91.5	Lamprinou et al. (2009)
Koutouki cave, Attica, Greece – L	0.02–0.035	15.7–16.5	92.3–95.1	Lamprinou et al. (2011)
Gelada cave, SE Spain – L	0.001–0.06	5.4–18	55–95	Martınez and Asencio (2010)
L'Aigua cave, SE Spain – L	0.3–(1,254)	15–29.4	24.7–81.5	Beltrán and Asencio (2009)
Cave of Bats, Zuheros, SW Spain – L	0.05–3.8 (18)	8.2–14.4	54–93.8	Urzì et al. (2010)
Catacomb of St. Callistus, Rome, Italy – P	0.01–0.15	18–20	94–95	Albertano and Bellezza (2001)
Catacomb of St. Domitilla, Ipogeo dei Flavi, Rome, Italy – T	0.05–2	17–22	87.5–97.6	Cadel and Albertano (unpublished)
Catacomb of Priscilla, Rome, Italy – T	0.2–0.6	16.5–17	99.0–99.9	Albertano and Urzì (1999)

L limestone, P wall paintings, T tufa rock. Values in brackets refer to the entrance areas

higher (2.44 μg cm^{-2}) than that of the epilithic assemblages at the cave entrance (up to 1.71 μg cm^{-2}). Comparisons of values for the cyanobacterial contribution to phototrophic diversity in caves gave remarkably similar values in Belgium (54%) and Germany (55%) to those in Slovenia (51%) (Mulec et al. 2008). The extensive studies of Slovenian karst caves reported a total of 197 cyanobacterial species.

In a study of the lampenflora of caves in Moravia, Czech Republic, about 20 cyanobacteria species contributed to 31% of the phototrophic diversity at one site (Poulíčková and Hašler 2007). Beltrán and Asencio (2009) showed that among the thirteen epilithic and chasmoendolithic cyanobacteria colonizing the walls of L'Aigua cave, S-E Spain, the proportion were 54% Chroococcales, 23% Oscillatoriales and 23% Nostocales. *Calothrix elenkinii, Gloeothece confluens* and *Hormothece cylindrocellulare* were observed for the first time in caves, along with chasmoendolithic growths of *Aphanothece saxicola, Gloeothece confluens* and *Pleurocapsa minor*. The values for the Gelada Cave were 77% Chroococcales, 14% Oscillatoriales, 4.5% Nostocales and 4.5% Stigonematales (Martınez and Asencio 2010). Of the 22 species identified, the most common were *Asterocapsa divina*, *Leptolyngbya leptotrichiformis* and *Scytonema julianum*. A decrease of Chroococcales and increase of Oscillatoriales from the entrance to the end of the cave has also been observed in caves of Israel (Vinogradova et al. 1998, 2009).

11.4.2 Light

The characteristically extremely low values for photosynthetic photon flux density (PPFD) of caves and other underground archaeological and historical sites (Table 11.2) can be changed when energy is introduced in the environment as occurs when lighting systems are installed. Variable regimes of natural and artificial light, temperatures and air humidity have been recorded in caves in France (Leclerc et al. 1983), Italy (Albertano and Urzì 1999; Hernández-Mariné et al. 2003), Slovenia and Spain (Asencio and Aboal 1996, 2000b). At the L'Aigua cave mentioned above, cyanobacterial growth occurred within specific ranges of fluctuation of PPFD and relative humidity (Beltrán and Asencio 2009). In the Gelada Cave the availability of light was the primary stress factor, followed by humidity, lack of nutrients and temperature (Martınez and Asencio 2010). Changes in local air currents caused by warming in the proximity of lamps can also favour microbial growth. Mulec and Kosi (2009) showed the influence of lighting on relative humidity in Slovenian caves with falls from 95% to 73% at 20 cm from a lamp, while temperature could increase by 8°C at 50 cm from a light source.

The depth to which phototrophic biofilms extend into the cave interior depends on the light gradients and, more precisely, on the amount of light at various wavelengths available for primary production (Albertano and Bruno 2003). In spite of the restricted emission in the visible part of the spectrum of the lamps used for lighting, numerous troglophilic cyanobacteria have adapted to the particular light conditions and contribute most of the phototrophic biomass (Table 11.3 and Fig. 11.7). The light available for photosynthesis in the inner part of caves and hypogea is usually restricted to periods when visitors are present and the cyanobacterial species composition is mostly determined by the available light (Albertano 1993). In the catacombs of Rome, the illuminated areas supporting phototrophic growth usually extend to around 1.0 m from the lamp. Although the floristic composition was not related clearly to decreasing irradiance, there was a trend for phototroph diversity to decrease under lower irradiances, with the biofilms at the lowest PPFD being uniquely built by erect filaments of *Leptolyngbya* (Hernández-Mariné et al. 2003).

Caves and other types of confined environment open to the public increasingly suffer due to the increases in air temperature, relative humidity and carbon dioxide concentration. The visitor influx also favours the condensation of water, mostly on vaults and the upper part of walls and in turn this all favours photosynthetic carbon fixation (Pulido-Bosch et al. 1997; Sanchez-Moral et al. 2005). The presence of atmospheric pollutants from urban and industrial emissions, namely CO_2 and NO_x, might enhance the growth of biofilms. Microbial diversity in caves can also be greatly

Table 11.3 Some of the cyanobacterial species recently reported for subterranean sites of the Mediterranean area

Species	References
Aphanocapsa muscicola	Lamprinou et al. (2009)
Aphanocapsa parietina	Roldán and Hernández-Mariné (2009), Urzì et al. (2010)
Aphanothece saxicola	Beltrán and Asencio (2009), Martınez and Asencio (2010)
Asterocapsa divina	Aboal et al. (2003), Martınez and Asencio (2010)
Calothrix elenkinii	Beltrán and Asencio (2009)
Chlorogloea microcystoides	Imperi et al. (2007)
Chroococcidiopsis doonensis	Asencio and Aboal (2000a), Lamprinou et al. (2009)
Chroococcus lithophilus	Imperi et al. (2007)
Chroococcus minor	Tomaselli et al. (2000), Macedo et al. (2009)
Chroococcus spelaeus	Lamprinou et al. (2009), Martınez and Asencio (2010)
Chroococcus turgidus	Lamprinou et al. (2009)
Chroococcus westii	Martınez and Asencio (2010)
Cyanobacterium cedrorum	Martınez and Asencio (2010)
Cyanosaccus aegeus	Martınez and Asencio (2010)
Cyanosaccus atticus	Martınez and Asencio (2010)
Cyanostylon gelatinosus	Albertano and Bellezza (2001)
Cyanostylon microcystoides	Martınez and Asencio (2010)
Eucapsis terrestris	Albertano and Bellezza (2001)
Fischerella maior	Albertano and Urzì (1999)
Geitleria calcarea	Abdelahad and Bazzichelli (1988), Ariño et al. (1997)
Gloeocapsa alpina	Urzì et al. (2010)
Gloeocapsa biformis	Asencio and Aboal (2000a), Imperi et al. (2007), Lamprinou et al. (2009), Martınez and Asencio (2010)
Gloeocapsa kuetzingiana	Imperi et al. (2007)
Gloeocapsa nigrescens	Martınez and Asencio (2010)
Gloeocapsa novacekii	Martınez and Asencio (2010)
Gloeocapsa rupestris	Imperi et al. (2007)
Gloeocapsopsis magma	Roldán and Hernández-Mariné (2009)
Gloeocasa rupicola	Martınez and Asencio (2010)
Gloeothece confluens	Beltrán and Asencio (2009)
Gloeothece membranacea	Albertano and Bellezza (2001), Bellezza and Albertano (2003)
Gloeothece rupestris	Imperi et al. (2007)
Herpyzonema pulverulentum	Hernández-Mariné and Canals (1994), Albertano et al. (2003)
Hormothece cylindrocellulare	Beltrán and Asencio (2009)
Iphinoe spelaeobios	Lamprinou et al. (2011)
Leptolyngbya carnea	Martınez and Asencio (2010)
Leptolyngbya gracillima	Asencio and Aboal (2000a), Albertano and Bellezza (2001), Lamprinou et al. (2009)
Leptolyngbya leptotrichiformis	Martınez and Asencio (2010)
Leptolyngbya sp. Green	Bruno et al. (2009)
Leptolyngbya sp. Red	Bruno et al. (2009), Martınez and Asencio (2010)
Loriella osteophila	Albertano et al. (2003), Bellezza et al. (2005)
Loriellopsis cavernicola	Lamprinou et al. (2011)
Myxosarcina	Abdelahad (1989)
Phormidium molle	Asencio and Aboal (2000a), Lamprinou et al. 2009,
Pleurocapsa minor	Beltrán and Asencio (2009), Martınez and Asencio (2010)
Pseudocapsa dubia	Imperi et al. (2007), Lamprinou et al. (2009), Martınez and Asencio (2010)
Scytonema julianum	Pietrini and Ricci (1993), Aboal et al.(1994), Ariño et al. (1997), Albertano and Urzì (1999), Lamprinou et al. (2009), Roldán and Hernández-Mariné (2009), Martınez and Asencio (2010)
Scytonema ocellatum	Albertano and Urzì (1999)
Symphyonema cavernicolum	Asencio et al. (1996), Martınez and Asencio (2010)

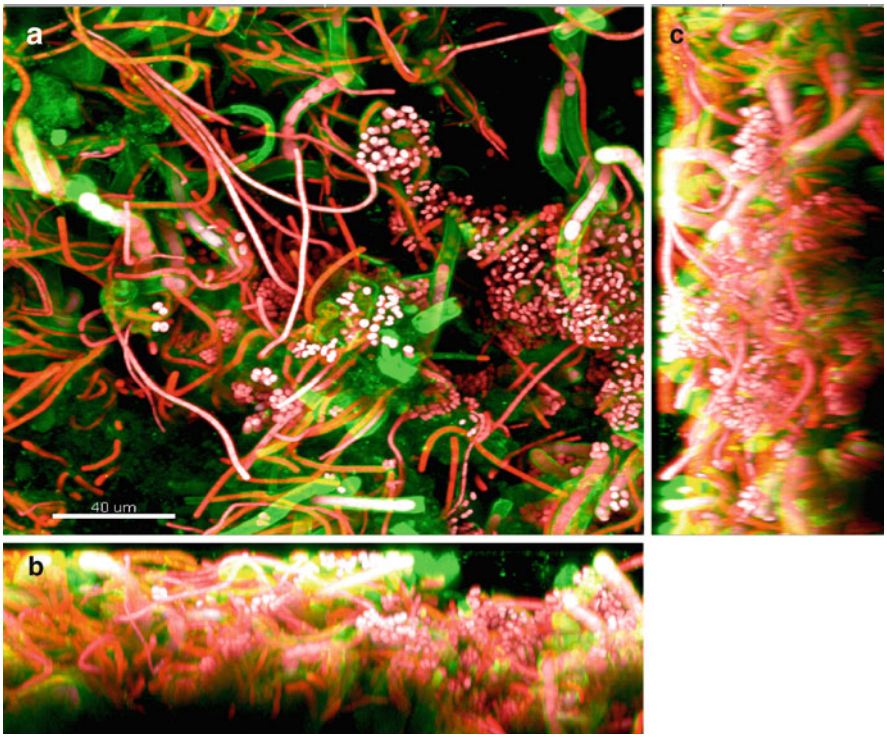

Fig. 11.7 Epilithic biofilm structure: (**a**) Coccoid and filamentous cyanobacteria with different morphologies and various autofluorescence due to the photosynthetic pigments provide a picture of the phenotypic diversity within biofilms as shown in confocal laser scanning microscopy by bi-channel extended-focus projection images (*section mode*) that allow lateral view in XZ (**b**) and YZ (**c**) of the community layering. Bar = 40 μm

affected by biocidal treatments in combination with other factors resulting from human activity.

11.4.3 The Organisms and Their Response to the Environment

In addition to the floristic information included in the reports mentioned above, there are a number of other detailed accounts of cyanobacteria adapted to extremely low PPFD values. These include ones about wall paintings in Italy (Nugari et al. 2009) and Malta (Zammit et al. 2011a), sinkholes and caves in Spain (Hernández-Mariné et al. 2001; Asencio and Aboal 2001, 2004; Roldán et al. 2004; Uher et al. 2005; Urzì et al. 2010), Greece (Lamprinou et al. 2009), Israel (Cor and Dor 1999) and Kentucky, USA (Smith and Olson 2007). Subterranean sites are revealing previously undescribed forms, such as *Leptolyngbya* species (Bruno et al. 2009) and several Stigonematales (Asencio et al. 1996; Lamprinou et al. 2011). The latter include *Iphinoe spelaeobios* gen. nov., sp. nov, in two Greek caves, *Loriellopsis cavernicola* gen. nov., sp. nov. in a cave in Catalonia, and *Symphyonema cavernicolum* sp. nov in Alicante, Spain. Different OTUs have also been described from the palaeo-Christian catacombs of St Agatha and St Paul at Rabat in Malta and trees have been constructed using 16S rRNA gene sequences as a step towards clarifying their taxonomic position (Zammit et al. 2010).

Observations on the ultrastructure of chasmoendolithic *Chroococcidiopsis*, *Cyanosarcina*, *Leptolyngbya*, *Phormidium* and *Pseudocapsa* in cave cyanobacteria in the Murcia region of S-E. Spain (Asencio and Aboal 2004) showed wide sheaths, a well developed thylakoid system and examples of the all the most widely known cell inclusions (carboxysomes, glycogen and cyanophycin granules, lipid globules, polyphosphate and poly-β-hydroxybutyric bodies). During a study on the morphological and ultrastructural variability of a red *Leptolyngbya* species from a site in Rome and *Rhabdogloea brasilica* from Brazilian caves using a temperature – light cross-gradient system, the values for both light and temperature optima were found to differ than those recorded *in situ* (Albertano and Kovacik 1996; Azevedo and Kováčik 1996). One remarkable feature of the red *Leptolyngbya* strain VRUC 135, very recently described as *Oculatella subterranea* gen. nov., sp. nov (Zammit et al. 2012), is its phototactic activity and the presence of an eye-spot like structure made of carotenoid globules and rhodopsin-like pigment at the tip of the apical cell (Albertano et al. 2000a). This complex photoreceptive structure may have an evolutionary importance since it resembles that observed in chloroplast of green flagellates.

Physiological adaptations of cyanobacteria to light limitation in caves and hypogea have seldom been studied. However, Mulec et al. (2008) reported a general increase of chlorophyll *a* and phycobiliprotein contents with decreasing light, features which are widely applicable to cyanobacteria in general. The light saturation values for cyanobacteria in culture range between 50 and 100 μmol photon m^{-2} s^{-1}, with the typical compensation point at about 5–6 μmol photon

m^{-2} s^{-1}. Nevertheless the latter values are higher than PPFD values measured at most sites, as has been shown for a *Phormidium* sp. living in the Frasassi caves, Italy, where the irradiance needed to compensate for respiration, 13 µmol photon m^{-2} s^{-1}, exceeded the available light, 6–10 µmol photon m^{-2} s^{-1} (Giordano et al. 2000). Nevertheless, phototrophic biofilms have been found on the frescoes of the Domus aurea in Rome at PPFD <0.05 µmol photon m^{-2} s^{-1} with a phycobiliproteins: chlorophyll *a* ratio >4 (Albertano and Grilli Caiola 1989). Most phototrophic biofilms in Roman catacombs develop at <2 µmol photon m^{-2} s^{-1} (Albertano and Urzì 1999; Albertano and Bellezza 2001). Amperometric measurement with oxygen microelectrodes generally showed high photosynthetic efficiency, photoinhibition at >200 µmol photon m^{-2} s^{-1} and confirmed low photosynthetic maxima and high respiration rates, possibly due to the heterotrophic associated bacteria (Compagnone et al. 1999). These biofilms, in culture at PPFD ten times those *in situ,* showed sheath thickening and a reduced amount of glycogen in dominant green *Leptolyngbya* species; filamentous bacteria were closely associated with the cell wall of the cyanobacterium (Albertano et al. 1991). Photosynthetic light saturation values <60 µmol photon m^{-2} s^{-1} have been measured in most of the strains isolated from the Domus aurea and catacombs of Rome including the red ex *Leptolyngbya* (Oculatella subterranea) species after acclimation of cultures to PPFD <10 µmol photon m^{-2} s^{-1} (Bruno and Albertano 1999). These strains possessed high number of phycobilisomes and thylakoids, and high phycobilin to chlorophyll ratios (between 10 and 17) (Bruno and Albertano 1999). Adaptation to cave habitats also includes the ability to use organic sources and grow hetetrophically (Adhikary 2002). *Scytonema coactile*, isolated from the twilight zone of an Indian cave and studied under different light – dark regimes, could use several exogenous sugars for heterotrophic and photoheterotrophic growth (Lakshmi et al. 2008); there was no requirement for protein induction before growth could occur in the dark.

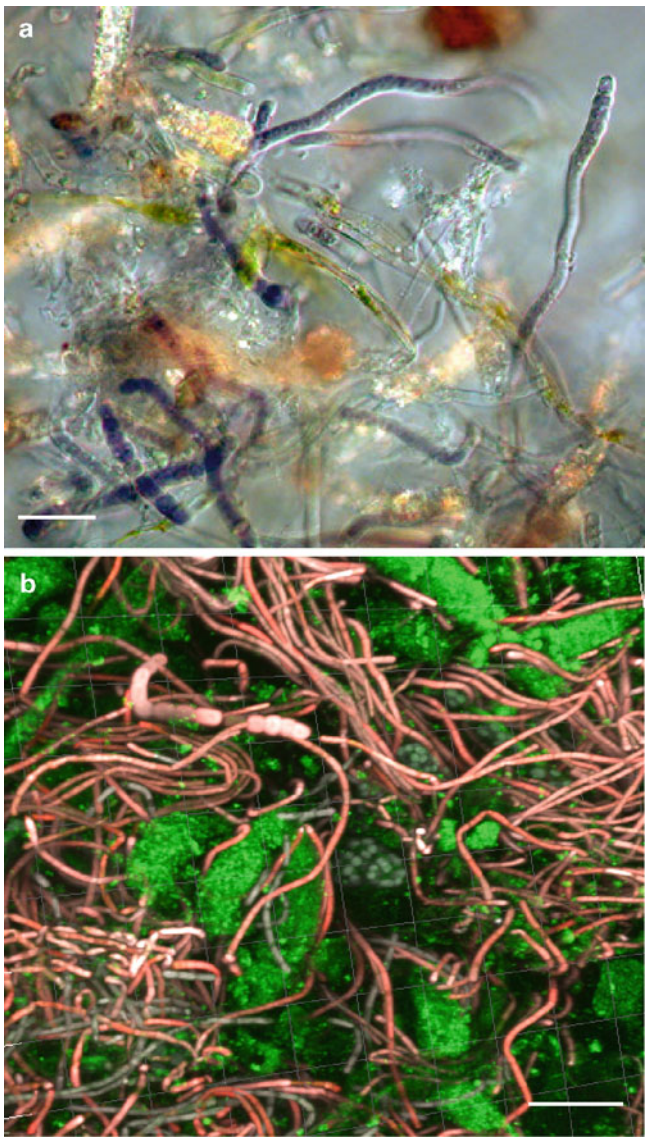

Fig. 11.8 Cyanobacterial communities in low light habitat: (**a**) Light microscopy of a biofilm fragment), with phycoerithryn-rich and calcifying filamentous species; (**b**) Confocal laser scanning microscopy of a *S. julianum* (calcite in green) and thin *Leptolyngbya* (pink) dominated community from St. Domitilla catacombs in Rome (Italy). Bars = 20 µm

11.4.4 Calcification in Subterranean Sites

Deposition of calcium carbonate on cyanobacterial filaments has been frequently observed in subterranean sites. *Scytonema julianum* is one of the calcifying species able to mobilize calcium ions from mineral substrata, as are *Geitleria calcarea*, *Herpyzonema pulverulentum* and *Loriella* sp. (Hernández-Mariné and Canals 1994; Ariño et al. 1997; Hernández-Mariné et al. 1999) and some *Leptolyngbya* and *Fischerella* spp. (Albertano 1997) (Fig. 11.8).

Scytonema julianum can form an extensive cover on lithic faces with a grey-greenish crusty or powdery surface alterations; it prefers rocks that are rich in calcium carbonate, but never wet. The areas colonized are usually well protected from air currents and characterized by high relative humidity (72–100%) and PPFD up to 8 µmol photon m^{-2} s^{-1}. Since it was first reported in the Roman Catacombs of Rome in 1992 (Ortega et al. 1993), it has been found on the Roman mortars of the Carmona necropolis, Spain (Ariño et al. 1997), in the rock church of Matera, Italy (Pietrini and Ricci 1993), and in several natural caves and archaeological hypogea (Albertano and Urzì 1999; Garbacki et al. 1999; Tomaselli et al. 2000; Cañaveras et al. 2001; Sanchez-Moral et al. 2005). In the sheath of *S. julianum* both acidic (glucuronic and galacturonic acids) and sulphated polysaccharides have been shown

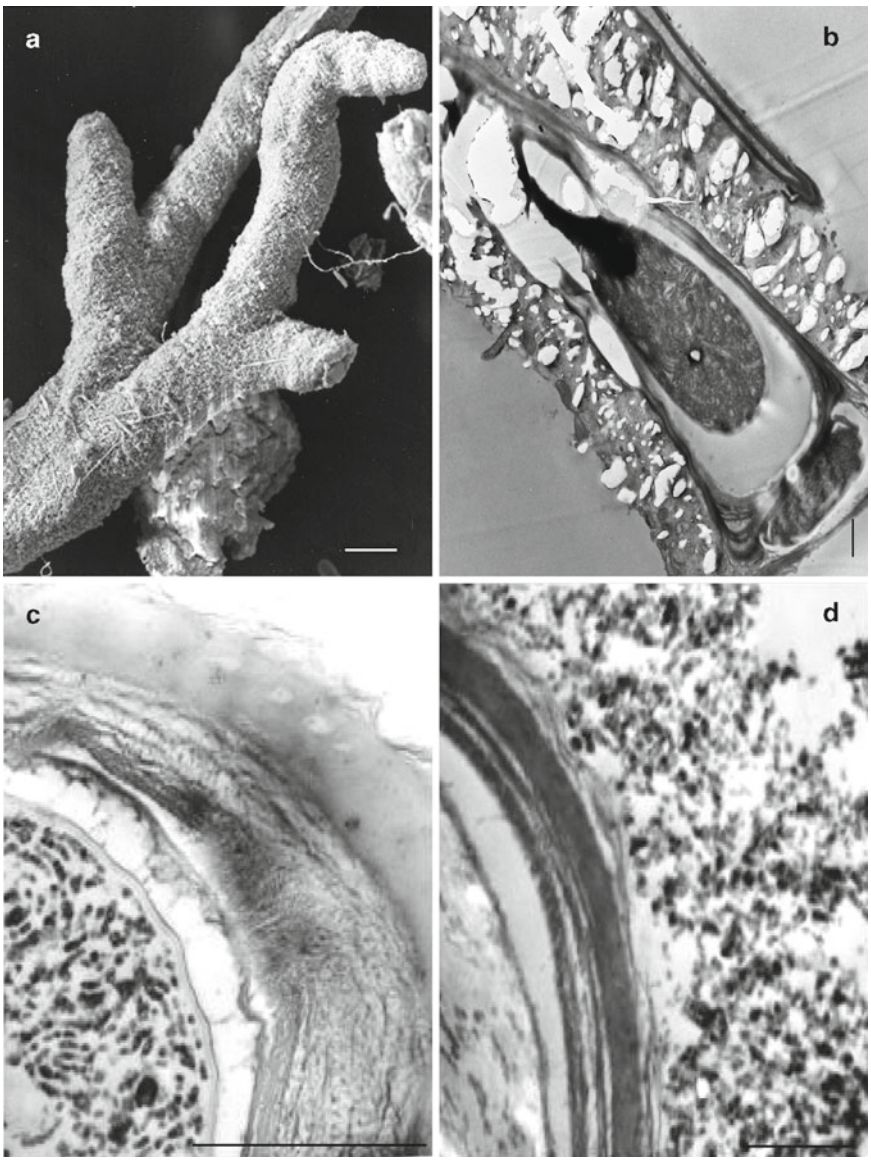

Fig. 11.9 Calcifying cyanobacteria: (**a**) Scanning electron microscopy view of the true-branching filaments of *Loriella* with mineralised sheath; (**b**) Transmission electron microscopy view of a longitudinal section of *Scytonema julianum* showing calcite crystals deposited within the outermost sheath layer**;** (**c**, **d**) Details of the by-layered sheath in cross-section after cytochemical stain to evidence the presence of polysaccharides throughout the sheath thickness (**c**) and glycoproteins (**d**) in the innermost part. Bars = 5 μm (**a**), 1 μm (**b–d**)

by cytochemistry, and further confirmed by chromatography and circular dichroism (Albertano and Bellezza 2001; Bellezza et al. 2005), wilst *S. ocellatum* and *Fischerella maior* isolates lacked glucuronic acid (Bellezza et al. 2006). The sheath of *S. julianum* in samples from Roman catacombs appeared to be organised in a thick diffluent outer layer rich in acidic polysaccharides and impregnated by calcite crystals, and an inner dense layer with a complex substructure (Fig. 11.9) (S. Cadel and the author, unpublished data). Negatively charged carboxylic and sulphated groups and positively charged glycoproteins made up the outer mucilage in which triradiate calcite crystals (Hoffmann 2002; Ariño et al. 1997) and anastomosing crystals (Aboal et al. 1994) could be observed. The crystals at the sheath surface probably undergo a repeated calcification/decalcification process depending on changes in pH, CO_2 species and calcium concentration (Riding 2006). Similarly, incrustation of calcite on *Geitleria calcarea* may be controlled by the organism itself (Pentecost and Whitton 2000). Indeed, photosynthesis linked alkalization has been measured using potentiometric microsensors within cyanobacterial biofilms during illumination with pH shifts above neutrality, sufficient to induce precipitation of carbonates (Albertano et al. 2000b). Precipitation of mineral particles on cells can lead epilithic strains to become endolithic (Asencio and Aboal 2001).

The preference for calcareous substrata is well documented also for *Loriellopsis cavernicola* (previously reported as *Loriella* sp. by Hernández-Mariné et al. 1999) in Spanish caves (Lamprinou et al. 2011). However, PPFD values supporting the development of *Loriella osteophila* in Roman catacombs (Fig. 11.9a) were lower, 0.05–2 μmol photon m^{-2} s^{-1}, than for *S. julianum*, which inhabits more illuminated

areas in the catacombs of St Domitilla in Rome (Table 11.2). *Loriella* also secretes a bilayered sheath characterized by complex polysaccharides, carboxylic and sulphated groups and glycoproteins, which are positively charged (S. Cadel and author, unpublished data), and similarly to *Loriellopsis*, both the inner and outer sheath layers are densely covered by crystals in shape of acinose granules or little sticks (Hernández-Mariné et al. 1999).

The presence of carboxylic and sulphated groups and glycoproteins positively charged in the capsular polysaccharides of the red *Leptolyngbya* species and *Fischerella maior* (Bellezza et al. 2003, 2005) adds further support to the suggested role of EPS in calcium accumulation in the sheath, which can favour the mineralisation processes in combination with the photosynthetic accumulation of OH^- (Riding 2006).

11.5 Colonization of Stone

Monuments can be considered as pristine environments that soon after their creation (as well as after each cleaning intervention) are exposed to several biophysical and biochemical processes that start with the adhesion of epilithic and endolithic organisms. Because of the autotrophic nature and ecological role of photosynthetic microorganisms, cyanobacteria, microalgae, mosses and lichens play a particular role in the deterioration of stone surfaces.

Passive dispersal of viable cyanobacteria and algae by means of water, air and other organisms is therefore a prerequisite to establish an active population at a particular site. In subaerial environments most microorganisms are transported by air and settle on surfaces where they can grow into biological patinas, the biofilms, where suitable conditions are suitable. Airborne cells and spores of microorganisms together with pollens and other biological particles attach to the exposed surfaces. Since Round (1981, as quoted in Kumar et al. 2007), remarked that there was no information on transport mechanism of algae, their dispersal mechanisms has occasionally received attention. The review by Kumar et al. (2007) about airborne algae reported 32 cyanobacterial species and gave an outline of the environmental factors that control aerosolization and transport, along with the biogeographical implications. The authors indicated that repeated cycles of drying/wetting favour the exfoliation of phototrophic biofilms during the dry period and the transport of airborne cyanobacterial cells spores from the soil by ascending currents to the atmosphere at distances up to 10 km. In confined environments the limited air circulation favours an increase of particle concentration and the chances of settlement on surfaces.

Biofilm development starts when microorganisms adhere to a surface, though adhesion mechanisms vary depending on the organism and substrate. The successful development of a species is determined by the nature and properties of mineral constituents of the substratum, its pH, salinity, water content, texture and porosity, and by environmental factors as temperature, relative humidity, light conditions, atmospheric pollution levels, wind, and rainfall. Prieto and Silva (2005) assessed potential bioreceptivity of granite varieties before exposure to specific environmental conditions, by measuring intrinsic rock properties such as abrasion pH, bulk density, open porosity and capillary water. All these properties were used to evaluate differences in the rock/water interactions, and hence in the bioreceptivity of granite to cyanobacteria during a 2-months experiment in growth chambers. Restoration treatments can favour microbial colonization when carried out without the appropriate methods and microbiological knowledge, since they may involve the use of inorganic (even water) or organic compounds that can support the growth of autotrophs or heterotrophs (Bastian and Alabouvette 2009).

As soon as microorganisms grow and divide, EPS secretion provides them with a protective highly hydrated matrix, which contributes to an increase in the bulk volume of the biofilm. This dynamic community can then spread across surfaces and incorporate other microorganisms. The production and composition of EPS seem to be remarkably variable depending on the microorganisms and nutritional conditions.

Gliding hormogonia and photoreception, as in red *Leptolyngbya* (Oculatella subterranea) species, allow effectives colonization of rock substrata. Motility and phototaxis have important roles in success under the low light conditions of the catacombs (Albertano et al. 2000a, b). Furthermore, the ability to secrete sheath-forming EPS rich in polysaccharides allowing adhesion can provide the strength sufficient to adhere to surfaces, as shown for subaerophytic green algae (Karsten et al. 2007). Alternatively, mucilage pads resulting from the lysis of necridic cells can remain attached to *Scytonema* hormocytes and act as extracellular spots for adhesion (Hernández-Mariné et al. 2001). Six years after restoration of the marbles of the statues in the Boboli Gardens, Florence, Italy, Lamenti et al. (2000) calculated a biofilm cover of 3×10^4 cells cm^{-2}, these consisting of a green alga and associated cyanobacteria. Laboratory studies on colonisation of stone by cyanobacteria have shown that in some cases appropriate culture conditions may allow reconstruction of natural communities from monuments to compare their capacity to colonize different lithotypes (Miller et al. 2006). Multidimensional and multispecies mathematical models, based on the theory of mixtures, are also developing to describe the cyanobacterial biofilm formation and the time evolution of live and dead cells and EPS on monument surfaces (Clarelli et al. 2009).

11.6 Interactions with Chemoorganotrophs

Although phototrophs are usually the first colonizers of bare rocks, the establishment of heterotrophic communities is possible without the pioneering participation of phototrophs, and may in turn sustain the subsequent growth of photosynthetic populations. In this case, organic matter naturally present in sedimentary rock (perhaps 0.2–2%), airborne particles, organic vapours, excreted metabolites and decaying biomass are used by the heterotrophs along with synthetic or natural organic substances from previous restoration treatments (Warscheid and Braams 2000).

Biodeterioration processes are rarely caused by a single group of microorganisms, and most often a synergistic effect on stone surfaces is achieved by the concomitant growth of phototrophic and heterotrophic populations. Bacteria and fungi can use the organic matter produced by phototrophs to release organic acids that dissolve the minerals of the substratum. Actinobacteria, microcolonial ascomycetous fungi and microscopic green algae are usually present in outdoor habitats, while spore-forming bacteria, troglobitic actinobacteria, diatoms and mosses form the subaerial biofilm adapted to low irradiance and high carbon dioxide (and other gases) concentrations. However, apart from the information on biofilms on stone monuments, little is still known about these associations. Scheerer et al. (2009) summarized data on the occurrence of previously unrecognized phototrophic bacteria related to *Chloroflexus* and Ectothiorhodospiraceae in samples at the Mayan site of Uxmal, and gave a short account of the contribution of these and other microorganisms to stone deterioration, their degradative role by acid/alkali production and by chelation along with the presence of chemoautotrophs (sulphur oxidizers, nitrifying bacteria, ferrous and manganous oxidizers).

In caves and confined environments, almost nothing appears to be known about fungi and their interactions with free-living phototrophs (Jurado et al. 2009), but different level of cyanobacterial association with bacteria have been observed *in situ* and in laboratory cultures. The adhesion of bacteria and penetration of actinobacteria into the mucilaginous sheath surrounding the trichomes of *Leptolyngbya* and *Scytonema* has been frequently observed, suggesting the establishment of a distinct syntrophism (Albertano and Urzì 1999). Strains of *Pseudomonas and Stenotrophomonas* have been recorded as the dominant airborne bacteria in the catacombs of St. Domitilla and St. Callistus in Rome (Saarela et al. 2004), and subsequently recognized in culture associations. Genome highly iterated octameric palindrome (HIP) sequences were used for PCR-fingerprinting of non-axenic *Leptolyngbya* strains maintained in culture for 11–20 years. These revealed the identity at genus and species levels of Gram-negative and Gram-positive bacteria from biofilms of the Roman catacombs from which the cyanobacteria had been isolated (Bruno et al. 2006). Other studies have shown non-culturable Acidobacteria as a relevant microbial component associated with cyanobacteria in many cave habitats and hypogea, including catacombs (Zimmerman et al. 2005, 2006). These cyanobacteria/bacteria relationships are regarded as a mutual-beneficial associations, where bacteria benefit from organic substances released from cyanobacteria and in return remineralized inorganic nutrients and carbon dioxide that can be used by the phototrophs. The previously unrecognized diversity of these subterranenean sites was also revealed by studies that allowed the detection of new species and the identification of several other taxa of bacteria associated with cyanobacteria (Urzì et al. 2008, 2010 and references therein).

Comparison of fresh and old collected biofilms from sandstone of the Bayon Temple in Angkor, Cambodia, showed that the bacterial community of old biofilm was very similar to the newly formed biofilm in terms of bacterial composition, but the eukaryotic communities were distinctly different between the two (Lan et al. 2010). Microscopy of biofilms from hindu temples in India has shown that Pseudomonadaceae and filamentous and rod-shaped actinobacteria are the most common heterotrophic prokaryotes associated with stress-tolerant cyanobacteria. Together with the fungi *Aspergillus*, *Cladosporium*, *Rhodorotula, Trichoderma* and *Ulocladium* they may represent a constant component of those tropical communities (C. Urzì, personal communication).

11.7 Methods for Studying Biodeterioration of Cyanobacterial Biofilms

The measurement of physical, chemical and climatic conditions in the microarea to be sampled, the recording of georeferenced coordinates and visual images of the position in the monument are the basic information needed to map subaerial biofilms on rocks and to understand the role they play in biodeterioration. In the field of cultural heritage the need for a multidisciplinary scientific approach is particularly evident. Archaeologysts, restorators and conservators continuously interact with researchers of scientific disciplines to set up dedicated methods, and discover and adapt new prevention and control measures for a specific monument.

To cope with the need of preserving man-made surface during the sampling of biofilms, techniques which do not increase the damage to the underlying substratum are much preferred (Zammit et al. 2008). Non-invasive techniques (NIT) for sampling are those which do not require a sample to be removed from the object and which essentially leave the object in the same state as before the analysis. Non-invasive sampling is undertaken by the application of adhesive tape strips (Gaylarde and Gaylarde 2000; Urzi and De

Leo 2001) and sterile humid paper filters to the surface to ensure the removal of biofilm and cells only. Non-destructive techniques (NDT) are used for analyses, while respecting the physical integrity of the sample. In addition, the removal of low-invasive micro-samples (1 × 1 mm) including a portion of the underlying substratum, can sometimes be permitted to investigate the interaction of the biofilm with the underlying substratum. This type of sample can subsequently be processed for structural and chemical analysis by non-destructive techniques using confocal laser scanning microscopy and environmental scanning electron microscopy coupled to Energy Dispersive X-Ray Spectroscopy (SEM-EDS), X-ray micro-fluorescence (μXRF) and X-ray micro-diffraction (μXRD) (Cuezva et al. 2009; Zammit et al. 2011b).

The application of light and electron microscopy to the study of biofilm-forming microbial communities is commonly used in the visualization of cyanobacterial biofilms, providing the means to characterize structural interactions between species, and resolving ultrastructural details at a micro- and nanometer scale. A variety of microscopy techniques (Fig. 11.7), are applied for the examination of microorganisms in the hypogean environment to assess their cytomorphological features and nutritional status (Albertano et al. 1991, 2003). Light microscopy epifluorescence, confocal laser microscopy scanning, and transmission electron microscopy allow to understand the organism relationships within the polymicrobial population and the interactions between mineral substrata and microorganisms by using micro-samples (Urzì and Albertano 2001).

These studies include the assessment of microbial diversity on surfaces. To improve conservation strategies relevant to the particular biofilm, with its structure and species, culture-dependent and culture-independent techniques are applied to study the genetic diversity of the biofilm. Because of the limitations of culture-based methods, molecular approaches such as those based on fluorescence in situ hybridization (FISH) techniques (La Cono and Urzì 2003) and those involving amplification of the 16S rRNA gene as universal phylogenetic markers are widely used to retrieve essential information on the structure of the communities. Thanks to the small amount of sample required by these approaches, denaturating gradient gel electrophoresis (DGGE) and single strand conformation polymorphism (SSCP) can be used to unravel the high diversity that cultural methods could resolve only poorly, and have become essential to investigate the microbial aetiology of the biodeterioration of monuments and art objects (Gonzalez and Saiz-Jimenez 2005; Miller et al. 2008, 2009; Macedo et al. 2009).

The convenience of combining 16S rRNA-based methods, i.e. ARDRA amplified rDNA restriction analysis and DGGE, with micro-Raman spectroscopy analysis for the identification of the aetiological agent(s) of a specific microbial-induced deterioration process on cave paintings has been reported by Hernanz et al. (2006) and Imperi et al. (2007). In many conservation laboratories, Raman spectroscopy has gained a leading position among standard techniques in the investigation of pigments from art and antiquities by virtue of its non-destructive nature and its ability to provide molecular information on a micrometer scale along with its applicability to both inorganic and organic substances.

Various methodological approaches have been developed to standardize rules and protocols for cultural heritage (Fassina 2008), along with specific methods to investigate phototrophic biofilms on stone (Albertano 2003; Albertano et al. 2003; Urzì and Albertano 2001).

Particularly important are the monitoring light detection and ranging (LIDAR) technique for the remote sensing of photoautotrophic biodeteriogens (Raimondi et al. 2009) and techniques based on the recording of visible light emission by portable spectroradiometry (Bruno et al. 2001; Albertano and Bruno 2003; Albertano et al. 2005), and spectral fingerprints (Roldán et al. 2004; Polerecky et al. 2009). The latter are usually applied to study the role of the spatial organization of microorganisms in the ecological functioning of complex microbial communities and for non-invasive monitoring of changes in the spatial organization and/or composition of a microbial community in response to environmental factors. Other spectral imaging systems have been developed for minimally invasive identification, localization, and relative quantification of pigments in cells and microbial communities. For pigment identification *in vivo* absorption and/or autofluorescence spectra are used as the analytical signals in CLSM.

The increasing interest in non-invasive approaches of monitoring and assessing damage and biodecay processes has boosted the use of microsensors to study changes in composition of chemical species mobilized upon stone surfaces during microbial metabolism. The growth of cyanobacterial films can induce more or less pronounced variation of the chemical parameters that characterize a microhabitat, and might possibly cause deleterious deteriogenic effects on the substrata. Amperometric oxygen microsensors were applied to measure photosynthesis and respiration in phototrophic biofilms that develop in Roman catacombs in order to record simultaneously curves of photosynthesis at increasing irradiance (P/I) (Compagnone et al. 1999), to measure phosphate concentration within biofilms (Calvo Quintana et al. 2004) and to apply potentiometric microelectrodes for the measurements of pH, K^+ and Ca^{2+} (Calvo Quintana et al. 2002). A decrease of potassium concentration occurred during light exposure in strains of *Scytonema julianum,* perhaps due to active uptake sustained by the photosynthetic activity. No appreciable decrease of soluble calcium due to the metabolic activity was observed in natural and artificial biofilms of the same species unless accumulation occurred in low active biofilms.

A final comment has to be made on the general approaches adopted in avoiding cyanobacterial growth on lithic faces. Among physical methods, monochromatic lamps resulted the most effective in reducing biofilm development in Roman catacombs (Albertano et al. 2005, 2007) and in Spanish cave (Roldán and Hernández-Mariné 2008). The established use of biocides, although with low toxicity, is still debated because of the unwanted effects on environment and human health, and in selecting microorganisms (Adhikary 2000a; Urzì and De Leo 2007; Bastian and Alabouvette 2009; Cappitelli et al. 2009; Nugari et al. 2003, 2009). In subterranean sites, a suitable combination of physical and chemical approaches can represent the best solution, while alternative methods may be based on the development and application of nanocomposites (Rodea-Palomares et al. 2010).

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References

Abdelahad N (1989) On four *Myxosarcin*a-like species (Cyanophyta) living in the Inferniglio cave (Italy). Arch Hydrobiol Suppl Algol Stud 54:3–13

Abdelahad N, Bazzichelli G (1988) *Geitleria calcarea* Friedmann, Cyanophycée cavernicole nouvelle pour l'Italie. Nova Hedwig 46:265–270

Aboal M, Ad A, Prefasi M (1994) Studies on cave cyanophytes from Southeastern Spain: *Scytonema julianum* Meneghini. Arch Hydrobiol Suppl Algol Stud 75:31–36

Aboal M, Ad A, Lopez-Jiménez E (2003) Morphological, ultrastructural and ecological study of *Asterocapsa divina* Komárek (Chroococcaceae, Cyanobacteria) from a cave of SouthEastern Spain. Arch Hydrobiol Suppl Algol Stud 109:57–65

Adhikary SP (2000a) Chlorophyll stability of epilithic cyanobacteria from temples of India. Arch Hydrobiol Suppl Algol Stud 98:119–131

Adhikary SP (2000b) Epilithic cyanobacteria on the exposed rocks and walls of temples and monuments of India. Indian J Microbiol 40:67–81

Adhikary SP (2002) Survival in darkness and heterotrophic growth of epilithic cyanobacteria from temples of India. Arch Hydrobiol Suppl Algol Stud 105:141–155

Adhikary SP (2004) Survival strategies of lithophytic cyanobacteria on the temples and monuments. In: Jain PC (ed) Microbiology and biotechnology for sustainable development. CBS Publishers and Distributors, New Delhi, pp 187–194

Adhikary SP, Satapathy DP (1996) *Tolypothrix byssoidea* (Cyanophyceae/Cyanobacteria) from temple rock surfaces of coastal Orissa, India. Nova Hedwig 62:419–423

Albertano P (1993) Epilithic algal communities in hypogean environments. G Bot Ital 127:386–392

Albertano P (1997) Elemental mapping as a tool in the understanding of microorganisms-substrate interactions. J Comp Assist Microsc 9: 81–84

Albertano P (2003) Methodological approaches to the study of stone alteration caused by cyanobacterial biofilms in hypogean environments. In: Koestler RJ, Koestler VR, Charola AE, Nieto-Fernandez FE (eds) Art, biology, and conservation: biodeterioration of works of art. The Metropolitan Museum of Art, New York, pp 302–315

Albertano P, Bellezza S (2001) Cytochemistry of cyanobacterial exopolymers in biofilms from Roman hypogea. Nova Hedwig 123: 501–518

Albertano P, Bruno L (2003) The importance of light in the conservation of hypogean monuments. In: Saiz-Jimenez C (ed) Molecular biology and cultural heritage. Swets and Zeitlinger BV, Lisse, pp 171–177, 287 pp

Albertano P, Grilli Caiola M (1989) A hypogean algal association. Braun Blanquetia 3:386–392

Albertano P, Kovacik L (1996) Light and temperature responses of terrestrial sciaphilous strains of *Leptolyngbya* sp. in cross-gradient cultures. Arch Hydrobiol Suppl Algol Stud 83:17–28

Albertano P, Urzì C (1999) Structural interactions among epilithic cyanobacteria and heterotrophic microorganisms in Roman hypogea. Microb Ecol 38:244–252

Albertano P, Luongo L, Grilli Caiola M (1991) Observations on cell structure of micro-organisms o fan epilithic phototrophic community competing for light. Nova Hedwig 53:369–381

Albertano P, Barsanti L, Passarelli V, Gualtieri P (2000a) A complex photoreceptive structure in the cyanobacterium *Leptolyngbya* sp. Micron 31:27–34

Albertano P, Bruno L, D'Ottavi D, Moscone D, Palleschi G (2000b) Effect of photosynthesis on pH variation in cyanobacterial biofilms from Roman catacombs. J Appl Phycol 12:279–384

Albertano P, Moscone D, Palleschi G, Hermosín B, Saiz-Jimenez C, Sánchez-Moral S, Hernández-Mariné M, Urzì C, Groth I, Schroeckh V, Saarela M, Mattila-Sandholm T, Gallon JR, Graziottin F, Bisconti F, Giuliani R (2003) Cyanobacteria attack rocks (CATS): control and preventive strategies to avoid damage caused by cyanobacteria and associated microorganisms in Roman hypogean monuments. In: Saiz-Jimenez C (ed) Molecular biology and cultural heritage. Swets and Zeitlinger BV, Lisse, pp 151–162, 287 pp

Albertano P, Bruno L, Bellezza S (2005) New strategy for the monitoring and control of cyanobacterial films on valuable lithic faces. Plant Biosyst 139:311–322

Albertano P, Bruno L, Piermarini S, Bellezza S (2007) Monochromatic light and portable spectroradiometry for the conservation of stone monuments affected by phototrophic micro-organisms. In: Drdacky M, Chapuis M (eds) Safeguarded cultural heritage – understanding and viability of the enlarged Europe, vol 2. Glos Semily, Praha, pp 814–817

Albertano P, Urzi' C, Caneva G (2009) Problems of biodeterioration in relation to particular types of environments. – Tombs, Catacombs and other Hypogea. In: Caneva G, Nugari MP, Salvadori O (eds) Plant biology for cultural heritage. Getty Conservation Institute, Los Angeles, pp 184–189

Ariño X, Hernández-Mariné M, Saiz-Jiménez C (1997) Colonization of Roman tombs by calcifying cyanobacteria. Phycologia 36:366–373

Asencio AD, Aboal M (1996) Cyanophytes from Andragulla cave (Murcia, SE Spain) and their environmental conditions. Arch Hydrobiol Suppl Algol Stud 83:55–72

Asencio AD, Aboal M (2000a) A contribution to knowledge of chasmoendolithic algae in cave-like environments. Arch Hydrobiol Suppl Algol Stud 98:133–151

Asencio AD, Aboal M (2000b) Algae from La Serreta cave (Murcia, SE Spain) and their environmental conditions. Arch Hydrobiol Suppl Algol Stud 96:59–78

Asencio AD, Aboal M (2001) Biodeterioration of wall paintings in caves of Murcia (SE Spain) by epilithic and chasmoendolithic algae. Arch Hydrobiol Suppl Algol Stud 103:131–142

Asencio AD, Aboal M (2004) Cell inclusions in the chasmoendolithic Cyanophytes from cave-like environments in Murcia (SE Spain). Arch Hydrobiol Suppl Algol Stud 113:117–127

Asencio AD, Aboal M, Hoffmann L (1996) A new cave-inhabiting blue-green alga: *Symphyonema cavernicolum* sp. nov. (Mastigocladaceae, Stigonematales). Arch Hydrobiol Suppl Algol Stud 83:73–82

Azevedo MT, Kováčik L (1996) *Rhabdogloea brasilica* sp. nov. (Chroococcales, Synechococcaceae): morphological and morphometric variability under cross-gradient cultures. Arch Hydrobiol Suppl Algol Stud 83:83–92

Barberousse H, Lombardo RJ, Tell G, Couté A (2006a) Factors involved in the colonisation of building façades by algae and cyanobacteria in France. Biofouling 22:69–77

Barberousse H, Tell G, Yéprémian C, Couté A (2006b) Diversity of algae and cyanobacteria growing on building façades in France. Arch Hydrobiol Suppl Algol Stud 120:81–105

Bastian F, Alabouvette C (2009) Lights and shadows on the conservation of a rock art cave: the case of Lascaux Cave. Int J Speleol Spec Issue Cave Microbiol 38:55–60

Bellezza S, Albertano P (2003) A Chroococcalean species from Roman hypogean sites: characterisation of *Gloeothece membranacea* (cyanobacteria, Synechoccaceae). Arch Hydrobiol Suppl Algol Stud 109:103–112

Bellezza S, Paradossi G, De Philippis R, Albertano P (2003) *Leptolyngbya* strains from Roman hypogea: cytochemical and physico-chemical characterisation of exopolysaccharides. J Appl Phycol 15:193–200

Bellezza S, Albertano P, De Philippis R, Paradossi G (2005) Exopolysaccharides in cyanobacterial biofilms from Roman catacombs. Arch Hydrobiol Suppl Algol Stud 117:117–132

Bellezza S, De Philippis R, Paradossi G, Albertano P (2006) Exopolysaccharides of two cyanobacterial strains from Roman hypogea. Geomicrobiol J 23:301–310

Beltrán JA, Asencio AD (2009) Cyanophytes from the L'Aigua cave (Alicante, SE Spain) and their environmental conditions. Algol Stud 132:21–34

Billi D (2009) Subcellular integrities in *Chroococcidiopsis* sp. CCMEE 029 survivors after prolonged desiccation revealed by molecular probes and genome stability assays. Extremophiles 13:49–57

Billi D (2012) Anhydrobiotic rock- inhabiting cyanobacteria: potential for astrobiology and biotechnology. In: Stan-Lotter H, Fendrihan F (eds) Adaptation of microbial life organisms in extreme environments: research and application. Springer, Wien New York, pp 119–132

Billi D, Potts M (2000) Life without water: responses of prokaryotes to desiccation. In: Storey KB, Storey JM (eds) Cell and molecular response to stress: environmental stressors and gene responses. Elsevier, Amsterdam, pp 181–192

Billi D, Potts M (2002) Life and death of dried prokaryotes. Res Microbiol 153:7–12

Billi D, Viaggiu E, Cockell CS, Rabbow E, Horneck G, Onofri S (2011) Damage escape and repair in dried *Chroococcidiopsis* spp. from hot and cold deserts exposed to simulated space and Martian conditions. Astrobiology 11:65–73

Bruno L, Albertano P (1999) Photoacclimation of sciaphilous epilithic cyanobacteria isolated from Roman hypogea. Arch Hydrobiol Suppl Algol Stud 94:89–103

Bruno L, Piermarini S, Albertano P (2001) Characterisation of spectral emission by cyanobacterial biofilms in the Roman Catacombs of Priscilla in Rome (Italy). Nova Hedwig 123:229–236

Bruno L, Billi D, Urzì C, Albertano P (2006) Genetic characterisation of epilithic cyanobacteria and their associated bacteria. Geomicrobiol J 23:293–299

Bruno L, Billi D, Bellezza S, Albertano P (2009) Cytomorphological and genetic characterization of troglophilic *Leptolyngbya* strains isolated from Roman hypogea. Appl Environ Microbiol 75:608–617

Büdel B (1999) Ecology and diversity of rock-inhabiting cyanobacteria in tropical regions. Eur J Phycol 34:361–370

Büdel B, Weber B, Kühl M, Pfanz H, Sültemeyer D, Wessels D (2004) Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. Geobiology 2:261–268

Büdel B, Bendix J, Bicker FR, Allan Green TG (2008) Dewfall as a water source frequently activates the endolithic cyanobacterial communities in the granites of Taylor Valley, Antarctica. J Phycol 44:1415–1424

Büdel B, Darienko T, Deutschewitz K, Stephanie Dojani S, Friedl T, Mohr KI, Salisch M, Reisser W, Weber B (2009) Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. Microb Ecol 57:229–247

Calvo Quintana J, Piermarini S, Albertano P, Palleschi G, Moscone D (2002) Potentiometric microsensors for cyanobacterial biofilms monitoring in hypogean environments. In: Di Natale C, D'amico A, Dori L, Cardinali GC, Nicoletti S (eds) Sensors and microsystems. World Scientific, River Edge, pp 270–274

Calvo Quintana J, Idrissi L, Palleschi G, Albertano P, Amine A, El Rhazi M, Moscone D (2004) Investigation of amperometric detection of phosphate. Application in seawater and cyanobacterial biofilm samples. Talanta 63:567–574

Cañaveras JC, Sanchez-Moral S, Soler V, Saiz-Jimenez C (2001) Microorganisms and microbially induced fabrics in cave walls. Geomicrobiol J 18:1521–1529

Caneva G, Nugari MP, Salvadori O (2009) Plant biology for cultural heritage. Getty Conservation Institute, Los Angeles, 408 pp

Cappitelli F, Abbruscato P, Foladori P, Zanardini E, Ranalli G, Principi P, Villa F, Polo A, Sorlini C (2009) Detection and elimination of cyanobacteria from frescoes: the case of the St. Brizio Chapel (Orvieto Cathedral, Italy). Microb Ecol 57:633–639

Castenholz RW, Garcia-Pichel F (2000) Responses to UV-radiation. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 591–611, 669 pp

Ciferri O (1999) Microbial degradation of paintings. Appl Environ Microbiol 65:879–885

Clarelli F, Di Russo C, Natalini R, Ribot M (2009) Mathematical models for biofilms on the surface of monuments. In: De Bernardis E, Spigler R, Valente V (eds) Advances in mathematics for applied science 82. World Scientific Publication, Singapore, pp 220–231

Compagnone D, Di Carlo V, Bruno L, Albertano P, Palleschi G (1999) Development of oxygen microsensor for monitoring cyanobacterial photosynthesis in Roman hypogea. Anal Lett 32:213–222

Crispim CA, Gaylarde CC (2005) Cyanobacteria and biodeterioration of cultural heritage: a review. Microb Ecol 49:1–9

Crispim CA, Gaylarde PM, Gaylarde CC (2003) Algal and cyanobacterial biofilms on calcareous historic buildings. Curr Microbiol 46:79–82

Cuezva S, Sanchez-Moral S, Saiz-Jimenez C, Canaveras JC (2009) Microbial communities and associated mineral fabrics in Altamira caves. Int J Speleol 38:83–92

Cuzman OA, Ventura S, Sili C, Mascalchi C, Turchetti T, D'Acqui LP, Tiano P (2010) Biodiversity of phototrophic biofilms dwelling on monumental fountains. Microb Ecol 60:81–95

Darienko T, Hoffmann L (2003) Algal growth on cultural monuments in Ukraine. Biologia 56:575–587

de los Rios A, Grube M, Sancho LG, Ascaso C (2007) Ultrastructural and genetic characteristics of endolithic cyanobacterial biofilms colonizing Antarctic granite rocks. FEMS Microbiol Ecol 59:386–395

De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22:151–175

Dobat K (1998) Flore de la lumiére artificiélle (lampenflora-maladie verte). In: Juberthie C, Deçu V (eds) Encyclopaedia Biospeleologica, Tome 2. Société de Biospéologie, Moulis-Bucarest, pp 1325–1335

Dor I, Dor Y (1999) Cyanobacterial flora of the Soreq stalactite Cave (Israel) and way of its control. Arch Hydrobiol Suppl Algol Stud 94:115–120

Fassina V (2008) European Technical Committee 346 – Conservation of Cultural Property – Updating of the activity after a three-year period. In: Notea A, Shoef Y (eds) Art 2008: non-destructive testing, microanalysis and preservation in the conservation of cultural and environmental heritage. ISAS International Seminars Ltd., Jerusalem, pp 1–9

Fleming ED, Castenholz RW (2007) Effects of periodic desiccation on the synthesis of the UVscreening compound, scytonemin, in cyanobacteria. Environ Microbiol 9:1448–1455

Foster JS, Green SJ, Ahrendt SR, Golubic S, Reid RP, Hetherington KL, Bebout L (2008) Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas. ISME J 3:573–587

Friedmann EI, Ocampo-Friedmann R (1984) Endolithic microorganisms in extreme dry environments: analysis of alithobiontic microbial habitat. In: Klug MJ, Reddy CA (eds) Microbial ecology. American Society for Microbiology, Washington, DC, pp 177–185

Fusey P, Hyvert G (1964) Les altérations physico-chimiques et biologiques des gres des monuments Khmers. Compt Rend Hebd Séances Groupes 13, 258(26):6573–6575 (in French)

Garbacki N, Ector L, Kostikov I, Hoffmann L (1999) Contribution à l'étude de la flore des grottes de Belgique. Belg J Bot 132:43–76

Gaylarde PM, Gaylarde CC (2000) Algae and cyanobacteria on painted buildings in Latin America. Int Biodeter Biodegr 46:93–97

Gaylarde PM, Gaylarde CC (2005) A comparative study of the major microbial biomass of biofilms on exteriors of buildings in Europe and Latin America. Int Biodeter Biodegr 55:131–139

Gaylarde CC, Morton LHG (2003) Biodeterioration of mineral materials. In: Bitton G (ed) Encyclopedia of environmental microbiology. Wiley, New York, pp 516–527

Gaylarde PM, Gaylarde CC, Guiamet P, Gomez de Saravia S, Videla H (2001) Biodeterioration of Mayan buildings at Uxmal and Tulum, Mexico. Biofouling 17:41–45

Giordano M, Mobili F, Pezzoni V, Hein MK, Davis JS (2000) Photosynthesis in the caves of Frasassi (Italy). Phycologia 39: 384–389

Golubic S (1967) Algenvegetation der Felsen, eine ökologische Algenstudie im dinarischen Karstgebiet. In: Elster HJ, Ohle W (eds) Die Binnengewässer, vol 23. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, p 183

Golubic S, Friedmann I, Schneider J (1981) The lithobiontic ecological niche, with special reference to microorganisms. J Sediment Petrol 5:475–478

Gonzalez JM, Saiz-Jimenez C (2005) Application of molecular nucleic acid-based techniques for the study of microbial communities in monuments and artworks. Int Microbiol 8:189–194

Gonzalez I, Laiz L, Hermosin B, Caballero B, Incerti C, Saiz-Jimenez C (1999) Bacteria isolated from rock art paintings: the case of Atlanterra shelter (South Spain). J Microbiol Methods 36:123–127

Gorbushina AA (2007) Life on the rocks. Environ Microbiol 9:1613–1631

Gorbushina AA, Broughton WJ (2009) Microbiology of the atmosphere-rock interface: how biological interactions and physical stresses modulate a sophisticated microbial ecosystem. Annu Rev Microbiol 63:431–450

Grbič ML, Vukojevič J, Subakov Simič G, Krizmanič J, Stupar M (2010) Biofilm-forming cyanobacteria, algae and fungi on two historic buildings in Belgrade, Serbia. Arch Biol Sci Belgrad 62:625–631

Grilli Caiola M, Billi D (2007) *Chroococcidiopsis* from desert to Mars. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 555–568

Hernández-Mariné M, Canals T (1994) *Herpyzonema pulverulentum* (Mastigocladaceae), a new cavernicolous atmophytic and lime-incrusted cyanophyte. Arch Hydrobiol Algol Stud Suppl 75:123–136

Hernández-Mariné M, Asencio AD, Canals A, Ariño X, Aboal M, Hoffmann L (1999) Discovery of populations of the lime-encrusting genus *Loriella* (Stigonematales) in Spanish caves. Arch Hydrobiol Algol Stud Suppl 94:121–138

Hernández-Mariné M, Roldán M, Clavero E, Canals A, Ariño X (2001) Phototrophic biofilm morphology in dim light. The case of the Puigmoltó sinkhole. Nova Hedwig 123:237–253

Hernández-Mariné M, Clavero E, Roldán M (2003) Why there is such luxurious growth in the hypogean environments. Arch Hydrobiol Suppl Algol Stud 109:229–240

Hernanz A, Mas M, Gavilán B, Hernández B (2006) Raman microscopy and IR spectroscopy of prehistoric paintings from Los Murciélagos cave (Zuheros, Córdoba, Spain). J Raman Spectrosc 37:492–497

Hoffmann L (1989) Algae of terrestrial habitats. Bot Rev 55:77–105

Hoffmann L (2002) Caves and other low-light environments: aerophytic photoautotrophic microorganisms. In: Bitton G (ed) Encyclopedia of environmental microbiology. Wiley, New York, pp 835–843

Imperi F, Caneva G, Laura Cancellieri L, Ricci MA, Sodo A, Visca P (2007) The bacterial aetiology of rosy discoloration of ancient wall paintings. Environ Microbiol 8:2894–2902

Jaag O (1945) Untersuchungen über die Vegetation und Biologie der Algen des näckten Gesteins in den Alpen, im Jura und im schweizerischen Mittelland. Beitr Kryptogamenflora Schweiz 9:1–560

Jurado V, Fernandez-Cortes A, Cuezva S, Laiz L, Canaveras JC, Sanchez-Moral S, Saiz-Jimenez C (2009) The fungal colonisation of rock-art caves: experimental evidence. Naturwissenschaften 96:1027–1034

Karsten U, Schumann R, Mostaert AS (2007) Aeroterrestrial algae growing on man-made surfaces. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 585–597, 811 pp

Kaštovsky J, Hauer T, Komárek J, Skácelová O (2010) The list of cyanobacterial species of the Czech Republic at the end of 2009. Fottea 10:235–249

Komárek J (2010) Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept). Hydrobiologia 639:245–259

Komárek J, Anagnostidis K (1998) Cyanoprokaryota I. Teil: Chroococcales. In: Ettl H, Gärtner G, Heynig H, Mollenhauer D (eds) Süsswasserflora von Mitteleuropa 19/1. Gustav Fischer, Jena/Stuttgart/Lübeck/Ulm, 548 pp

Komárek J, Anagnostidis K (2005) Cyanoprokaryota 2. Teil: Oscillatoriales. In: Büdel B, Krienitz L, Gärtner G, Schagerl M (eds) Süsswasserflora von Mitteleuropa 19/2. Elsevier GmbH, Spektrum Akademischer Verlag, Heidelberg, 759 pp

Komárek J, Hauer T (2010) CyanoDB.cz – On-line database of cyanobacterial genera. –World-wide electronic publication, University of South Bohemia and Institute of Botany ASCR. htpp://www.cyanodb.cz

Kovácik L (2000) Cyanobacteria and algae as agents of biodeterioration of stone substrata of historical buildings and other cultural monuments. In: Choi S, Suh M (eds) New millenium international forum on conservation of cultural property. Institute of Conservation Science for Cultural Heritage, Kongiu, pp 44–58

Kumar R, Kumar AV (1999) Biodeterioration of stone in tropical environments – an overview. J. Paul Getty Trust, Los Angeles, 85 pp

Kumar NS, Kumar R, Singh S, Brown RM Jr (2007) Airborne algae: their present status and relevance. J Phycol 43:615–627

La Cono V, Urzì C (2003) Fluorescent *in situ* hybridization (FISH) applied on samples taken with adhesive tape strips. J Microbiol Methods 55:65–71

Lakshmi KS, Adhikary SP (2008) Diversity of microalgae and cyanobacteria on building facades and monuments of India. Algae 23:91–114

Lakshmi KS, Jnanendra R, Adhikary SP (2008) Growth response and protein profile of two different *Scytonema* species from cave walls and soil crusts in light and dark. Algol Stud 127:49–60

Lamenti G, Tiano P, Tomaselli L (2000) Biodeterioration of ornamental marble statues in the Boboli Garden (Florence, Italy). J Appl Phycol 12:427–433

Lamprinou V, Pantazidou A, Papadogiannaki G, Radea C, Economou-Amilli A (2009) Cyanobacteria and associated invertebrates in Leontari Cave, Attica (Greece). Fottea 9:155–164

Lamprinou V, Hernández-Mariné M, Canals T, Kormas K, Economou-Amilli A, Pantazidou A (2011) Two new Stigonematalean cyanobacteria: *Iphinoe spelaeobios* gen. nov., sp. nov. and *Loriellopsis cavernicola* gen. nov., sp. nov. from Greek and Spanish caves. Morphology and molecular evaluation. Int J Syst Evol Microbiol. doi:10.1099/ijs.0.029223-0

Lan W, Hui Li H, Wang W-D, Katayama Y, Gu J-D (2010) Microbial community analysis of fresh and old microbial biofilms on Bayon Temple Sandstone of Angkor Thom, Cambodia. Microb Ecol 60:105–115

Leclerc JC, Couté A, Dupuy P (1983) Le climat annuel de deux grottes et d'une église du Poitou, ou vivent des colonies pures d'algues sciaphiles. Cryptogam Algol 4:1–19

Lefèvre M (1974) La maladie verte de Lascaux. Stud Conserv 19:126–156

Lefèvre M, Laporte G, Bauer J (1964) Sur les microorganismes envahissant les peintures rupestres de la grotte préhistorique de Lascaux. C R Acad Sci 258:5116–5118

Macedo MF, Miller AZ, Dioniso A, Saiz-Jimenez C (2009) Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview. Microbiology 155:3476–3490

Martınez A, Asencio AD (2010) Distribution of cyanobacteria at the Gelada Cave (Spain) by physical parameters. J Cave Karst Stud 72:11–20

May E, Jones M, Mitchell J (2008) Heritage microbiology and science: microbes, monuments and maritime materials. Cambridge RCS Publishing, Cambridge, 305 pp

McNamara CJ, Mitchell R (2005) Microbial deterioration of historic stone. Front Ecol Environ 3:445–451

McNamara CJ, Perry TD, Bearce KA, Hernandez-Duque G, Mitchell R (2006) Epilithic and endolithic bacterial communities in limestone from a Maya archaeological site. Microb Ecol 51:51–64

Miller A, Dioniso A, Macedo MF (2006) Primary bioreceptivity: a comparative study of different Portuguese lithotypes. Int Biodeter Biodegr 57:136–142

Miller AZ, Laiz L, Gonzalez JM, Dionísio A, Macedo MF, Saiz-Jimenez C (2008) Reproducing stone monument photosynthetic-based colonization under laboratory conditions. Sci Total Environ 405:278–285

Miller AZ, Laiz L, Dionísio A, Macedo MF, Saiz-Jimenez C (2009) Growth of phototrophic biofilms from limestone monuments under laboratory conditions. Int Biodeter Biodegr 63:860–867

Mohammadi P, Krumbein WE (2008) Biodeterioration of ancient stone materials from the Persepolis monuments (Iran). Aerobiologia 24:27–33

Mulec J, Kosi G (2009) Lampenflora algae and methods of growth control. J Cave Karst Stud 71:109–115

Mulec J, Kosi G, Vrhovšek D (2008) Characterization of cave aerophytic algal communities and effects of irradiance levels on production of pigments. J Cave Karst Stud 70:3–12

Nugari MP, Ricci S, Roccardi A, Monte M (2003) Churches and hypogea. In: Mandrioli P, Caneva G, Sabbioni C (eds) Cultural heritage and aerobiology, methods and measuring techniques for biodeterioration monitoring. Kluwer Academic Publishers, Dordrecht, pp 207–244

Nugari MP, Pietrini AM, Caneva G, Imperi F, Visca P (2009) Biodeterioration of mural paintings in a rocky habitat: the Crypt of the Original Sin (Matera, Italy). Int Biodeter Biodegr 63:705–711

Nuhoglu Y, Oguz E, Uslu H, Ozbek A, Ipekoglu B, Ocak I, Hasenekoglu I (2006) The accelerating effects of the microorganisms on biodeterioration of stone monuments under air pollution and continental-cold climatic conditions in Erzurum, Turkey. Sci Total Environ 364:272–283

Ortega-Calvo JJ, Hernández-Mariné M, Saiz-Jiménez C (1991) Biodeterioration of building materials by cyanobacteria and algae. Int Biodeter 28:165–185

Ortega-Calvo JJ, Hernandez-Mariné M, Saiz-Jimenez C (1993) Cyanobacteria and algae on historic building and monuments. In: Garg KL, Garg N, Mukerji KG (eds) Récent advances in biodeterioration and biodégradation, vol 1. Naya Prokash, Calcutta, pp 173–203

Ortega-Calvo JJ, Arifto X, Stal LJ, Saiz-Jimenez C (1994) Cyanobacterial sulfate accumulation from black crust of a historic building. Geomicrobiol J 12:15–22

Ortega-Calvo JJ, Ariño X, Hernandez-Mariné M, Saiz-Jimenez C (1995) Factors affecting the weathering and colonization of monuments by phototrophic microorganisms. Sci Total Environ 167:329–341

Ortega-Morales O (2006) Cyanobacterial diversity and ecology on historic monuments in Latin America. Rev Latinoam Microbiol 48:188–195

Ortega-Morales O, Guezennec J, Hernandez-Duque G, Gaylarde CC, Gaylarde PM (2000) Phototrophic biofilms on ancient Mayan buildings in Yucatan, Mexico. Curr Microbiol 40:81–85

Ortega-Morales O, Gaylarde CC, Englert GE, Gaylarde PM (2005) Analysis of salt-containing biofilms on limestone buildings of the Mayan culture at Edzna, Mexico. Geomicrobiol J 22:261–268

Pattanaik B, Adhikary SP (2002a) Blue green algal flora at some archaeological sites and monuments in India. Feddes Repert 113:289–300

Pattanaik B, Adhikary SP (2002b) Blue green algal flora on archaeological monuments of India. Bull Bot Surv India 44:61–74

Pattanaik B, Sahu JK, Adhikary SP (2004) Changes in the protein profile of cyanobacteria from terrestrial habitats in response to heat and UV-B radiation. Archiv Hydrobiol Algol Stud 113:175–182

Pattanaik B, Schumann R, Karsten U (2007) Effects of ultraviolet radiation on cyanobacteria and their protective mechanisms. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 31–45, 811 pp

Pentecost A (1992) Growth and distribution of endolithic algae in some North Yorkshire streams (UK). Br Phycol J 27:145–151

Pentecost A, Whitton BA (2000) Limestones. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publisher, Dordrecht, pp 257–279, 669 pp

Pereira S, Zille A, Micheletti E, Modaras-Ferreira P, De Philippis R (2009) Complexity of cyanobacterial exoploysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev 33:917–941

Pietrini AM, Ricci S (1993) Occurrence of a calcareous blue-green alga, *Scytonema julianum* (Kütz) Meneghini, on the frescoes of a church carved from the rock in Matera, Italy. Cryptogamic Bot 3:290–295

Pietrini AM, Ricci S (2009) Problems of biodeterioration in relation to particular types of environments. – Fountains and Nynphaea. In: Caneva G, Nugari MP, Salvadori O (eds) Plant biology for cultural heritage. Getty Conservation Institute, Los Angeles, pp 200–205

Pietrini AM, Ricci S, Nugari MP (2009) Churches and crypts. In: Caneva G, Nugari MP, Salvadori O (eds) Plant biology for cultural

heritage. Biodeterioration and conservation. Getty Conservation Institute, New York, pp 179–183
Polerecky L, Bissett A, Al-Najjar M, Faerber P, Osmers H, Suci PA, Stoodley P, de Beer D (2009) Modular spectral imaging system for discrimination of pigments in cells and microbial communities. Appl Environ Microbiol 75:758–771
Poulíčková A, Hašler P (2007) Aerophytic diatoms from caves in central Moravia (Czech Republic). Preslia 79:185–204
Prieto B, Silva B (2005) Estimation of the potential bioreceptivity of granitic rocks from their intrinsic properties. Int Biodeter Biodegr 56:206–215
Pulido-Bosch A, Martin-rosales W, Lopez-Chicano M, Rodriguez-Navarro CM, Vellejos A (1997) Human impact in a tourist karstic cave (Aracena, Spain). Environ Geol 31:142–149
Raimondi V, Cecchi G, Lognoli D, Palombi L, Grőnlund A, Johansen A, Svanberg S, BarupK HJ (2009) The fluorescence LIDAR technique for the remote sensing of photoautotrophic biodeteriogens in the outdoor cultural heritage: a decade of *in situ* experiments. Int Biodeter Biodegr 63:823–835
Raistrick A, Gilbert OL (1963) Malham Tarn House: its building materials, their weathering and colonization by plants. Field Stud Counc 1:89–115
Rajaniemi P, Hrouzek P, Kaštovská K, Willame R, Rantala A, Hoffmann L, Komárek J, Sivonen K (2005) Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). Int J Syst Evol Microbiol 55:11–26
Ramírez M, Hernández-Mariné M, Novelo E, Roldán M (2010) Cyanobacteria-containing biofilms from a Mayan monument in Palenque, Mexico. Biofouling 26:399–409
Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PS (2003) Bacterial co-aggregation: an integral process in the development of multi-species biofilms. Trends Microbiol 2:94–100
Riding R (2006) Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic–Cambrian changes in atmospheric composition. Geobiology 4:299–316
Rindi F (2007) Diversity, distribution and ecology of green algae and cyanobacteria in urban habitats. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 621–638, 811 pp
Rodea-Palomares I, Boltes K, Fernández-Piñas F, Leganés F, García-Calvo E, Santiago J, Rosal R (2010) Physicochemical characterization and ecotoxicological assessment of CeO_2 nanoparticles using two aquatic microorganisms. Toxicol Sci 119:135–145
Roldán M, Hernández-Mariné M (2008) The influence of green light on cyanobacterial fine structure: applicability for dim environments. Algol Stud 126:159–171
Roldán M, Hernández-Mariné M (2009) Exploring the secrets of the three-dimensional architecture of phototrophic biofilms in caves. Int J Speleol 38:41–53
Roldán M, Clavero E, Canals A, Gómez-Bolea A, Ariño X, Hernández-Mariné M (2004) Distribution of phototrophic biofilms in cavities (Garraf, Spain). Nova Hedwig 78:329–351
Roldan M, Oliva F, Gonzalez del Valle MA, Saiz-Jimenez C, Hernandez-Marine M (2006) Does green light influence the fluorescence properties and structure of phototrophic biofilms? Appl Environ Microbiol 72:3026–3031
Rossi F, Micheletti E, Bruno L, Adhykary SP, Albertano P, De Philippis R (2012) Characteristics and role of the exocellular polysaccharides produced by five cyanobacteria isolated from phototrophic biofilms growing on stone monuments. Biofouling 28:215–224
Roy A, Tripathy P, Adhikary SP (1997) Epilithic blue-green algae/cyanobacteria from temples of India and Nepal. II. Presence of UV sunscreen pigments. Arch Hydrobiol Algol Stud 86:147–161
Saarela M, Halakomi HL, Suihko ML, Maunuksela L, Raaska L, Mattila-Sandholm T (2004) Heterotrophic microorganisms in air and biofilm samples from Roman catacombs, with special emphasis on actinobacteria and fungi. Int Biodeter Biodegr 54:27–37
Saiz-Jimenez C, Garcia-Rowe J, Garcia del Cura MA, Ortega-Calvo JJ, Roekens E, van Grieken R (1990) Endolithic cyanobacteria in Maastricht limestone. Sci Total Environ 94:209–220
Salkinoja-Salonen MS, Peltola J, Andersson MA (2003) Microbial toxins in moisture damaged indoor environment and cultural assets. In: Saiz-Jimenez C (ed) Molecular biology and cultural heritage. Swets and Zeitlinger BV, Lisse, pp 93–105
Sánchez-Antón F, Asencio-Martínez AD (2007) Participation of Cyanophyceae in the biodeterioration of the stones of the Santo Domingo College in Orihuela, Alicante (SE Spain). Algol Stud 124:95–108
Sanchez-Moral S, Luque L, Cuezva S, Soler V, Benavente D, Laiz L, Gonzalez JM, Saiz-Jimenez C (2005) Deterioration of building materials in Roman catacombs: the influence of visitors. Sci Total Environ 349:260–276
Scheerer S, Ortega-Morales O, Gaylarde C (2009) Microbial deterioration of stone monuments -an updated overview. Appl Microbiol 66:97–139
Sigler WV, Bachofen R, Zeyer J (2003) Molecular characterization of endolithic cyantobacteria inhabiting exposed dolomite in central Switzerland. Environ Microbiol 5:618–627
Smith T, Olson R (2007) A taxonomic survey of Lamp Flora (Algae and Cyanobacteria) in electrically lit passages within Mammoth Cave National Park, Kentuky. Int J Speleol 36:105–114
Stal LJ (2000) Cyanobacterial mats and stromatolites. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 61–120, 669 pp
Stal LJ (2007) Cyanobacteria: diversity and versatility, clues to life in extreme environments. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 661–680, 811 pp
Stal LJ (2010) Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. Ecol Eng 36:236–245
Taton A, Grubisic S, Balthasart P, Hodgson DA, Laybourn-Parry J, Wilmotte A (2006) Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. FEMS Microbiol Ecol 57:272–289
Tomaselli L, Lamenti G, Bosco M, Tiano P (2000) Biodiversity of photosynthetic microorganisms dwelling on stone monuments. Int Biodeter Biodegr 46:251–258
Tripathi SN, Chung IK, Lee JA (2007) Diversity and characteristics of terrestrial cyanobacteria near Gimhae City, Korea. J Plant Biol 50:50–59
Tripathy P, Roy A, Adhikary SP (1997) Survey of epilithic blue-green algae (Cyanobacteria) from temples of India and Nepal. Arch Hydrobiol Suppl Algol Stud 87:43–57
Tripathy P, Roy A, Anand N, Adhikary SP (1999) Blue green algal flora on the rock surface of temples and monuments of India. Feddes Reper 110:133–144
Uher B, Aboal M, Kovacik L (2005) Epilithic and chasmoendolithic phycoflora of monuments and buildings in South-Estern Spain. Crypt Algol 26:275–358
Urzì C (2004) Microbial deterioration of rocks and marble monuments of the Mediterranean basin: a review. Corros Rev 22:441–459
Urzì C, Albertano P (2001) Studying phototrophic and heterotrophic microbial communities on stone monuments. Methods Enzymol 336:340–355. Academic Press, San Diego
Urzi C, De Leo F (2001) Sampling with adhesive tape strips: an easy and rapid method to monitor microbial colonization on monument surfaces. J Microbiol Methods 44:1–11
Urzì C, De Leo F (2007) Evaluation of the efficiency of water-repellent and biocide compounds against microbial colonization of mortars. Int Biodeter Biodegr 60:25–34
Urzì C, Krumbein WE, Warscheid T (1992) On the question of biogenic colour changes of Mediterranean monuments (coating, crust, micro-

stromatolite, patina, scialbatura, skin, rock varnish). In: Decrouez D, Chamay J, Zezza F (eds) Proceedings of the 3rd international symposium on the conservation of stone. Musée d'Art et d'Histoire, Genève, pp 397–420

Urzì C, De Leo F, Schumann P (2008) *Kribbella catacumbae* sp. nov. and *Kribbella sancticallisti* sp. nov., isolated from whitish-grey patinas in the Catacombs of St Callistus in Rome, Italy. Int J Syst Evol Microbiol 58:2090–2097

Urzì C, De Leo F, Bruno L, Albertano P (2010) Microbial diversity in Paleolithic caves: a study case on the phototrophic biofilms of the Cave of Bats (Zuheros, Spain). Microb Ecol 60:116–129

Uzunov BA, Stoyneva MP, Gärtner G (2007) Review of the studies on aero-terrestrial cyanoprokaryotes and algae in Bulgaria with a checklist of the recorded species.1. Phytol Balc 13:65–73

Vincent W (2007) Cold tolerance in cyanobacteria and life in the cryosphere. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 289–301, 811 pp

Vinogradova ON, Kovalenko OV, Wasser SP, Nevo E, Weinstein-Eviron M (1998) Species diversity gradient to darkness stress in blue-green algae/cyanobacteria: a microscale test in a prehistoric cave, Mount Carmel, Israel. Isr J Sci 46:229–238

Vinogradova ON, Nevo E, Wasser SP (2009) Algae of the Sefunum Cave (Israel): species diversity affected by light, humidity and rock stresses. Int J Algae 11:99–116

Warscheid T, Braams J (2000) Biodeterioration of stone: a review. Int Biodeter Biodegr 46:343–368

Wilmotte A (1994) Molecular evolution and taxonomy of the cyanobacteria. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Boston, pp 1–25

Wilmotte A, Herdmann M (2001) Phylogenetic relationships among the cyanobacteria based on 16S rRNA sequencing. In: Boone DR, Castenholz RW (eds) Bergey's manual of systematic bacteriology, vol I. Springer, New York, pp 487–493

Wynn-Williams DD (2000) Cyanobacteria in deserts – life at the limits? In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 341–366, 669 pp

Zakhis F, Jungblut A-D, Taton A, Vincent WF, Wilmotte A (2008) Cyanobacteria in cold ecosystems. In: Margesin R, Schinner F, Marx J-C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Dordrecht, pp 121–128

Zammit G, Psaila P, Albertano P (2008) An investigation into biodeterioration caused by microbial communities colonising Maltese Palaeo-Christian Catacombs. In: Notea A, Shoef Y (eds) Art 2008: non-destructive testing, microanalysis and preservation in the conservation of cultural and environmental heritage. ISAS International Seminars Ltd., Jerusalem, pp 1–10

Zammit G, Kastovsky J, Albertano P (2010) A first cytomorphological and molecular characterization of a new Stigonematalean cyanobacterial morphotype isolated from Maltese catacombs. Algol Stud 135:1–14

Zammit G, Billi D, Shubert ET, Kastovsky J, Albertano P (2011a) The biodiversity of subaeorophytic phototrophic biofilms from Maltese hypogea. Fottea 11:187–201

Zammit G, Sanchez-Moral S, Albertano P (2011b) Bacterially mediated mineralisation processes lead to biodeterioration of artworks in Maltese catacombs. Sci Total Environ 409:2773–2782

Zammit G, Billi D, Albertano P (2012) The subaerophytic cyanobacterium *Oculatella subterranea* (Oscillatoriales, Cyanophyceae) gen. et sp. nov: a cytomorphological and molecular description. Euro J Phycol 47 (in press)

Zanardini E, Abbruscato P, Ghedini N, RealiniM SC (2000) Influence of atmospheric pollutants on biodeterioration of stone. Int Biodeter Biodegr 45:35–42

Zimmerman J, Gonzales JM, Ludwig W, Saiz-Jimenez C (2005) Detection and phylogenetic relationships of highly diverse uncultured acidobacterial communities in Altamira cave using 23S rRNA sequence. Geomicrobiol J 22:379–388

Zimmerman J, Gonzales JM, Saiz-Jimenez C (2006) Epilithic biofilms in Saint Callixtus Catacombs (Rome) harbour a broad spectrum of Acidobacteria. Antonie Van Leeuwenhoek 89:203–208

Semi-arid Regions and Deserts

12

Chunxiang Hu, Kunshan Gao and Brian A. Whitton

Contents

C.X. Hu (✉)
State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, PR China
e-mail: cxhu@ihb.ac.cn

K. Gao
State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian 361005, PR China
e-mail: ksgao@xmu.edu.cn

B.A. Whitton
School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK
e-mail: b.a.whitton@durham.ac.uk

Summary

Phototrophic microorganisms are mostly endolithic or hypolithic in the more extreme arid environments and are here restricted to situations where sufficient moisture is retained for occasional growth to occur. Slightly less extreme environments frequently have biological soil crusts. In both cases cyanobacteria are the phototrophs most likely to be found and in some cases the only ones. In most cases of crust development *Microcoleus vaginatus* is one of the first cyanobacteria to occur. The crusts play an important role in maintaining soil and sand surfaces in arid regions, so it is important to understand how environmental factors influence communities at a site. In addition to light, water, temperature, salinity, nutrients and carbon dioxide, these include wind action and physical and chemical features of the underlying substratum. Experimental studies have confirmed that some species, such as the semi-desert *Nostoc flagelliforme*, are extremely resistant to damage by high light and UV levels. *N. flagelliforme* and at least some other species require a regular cycle of hydration and dehydration. Cyanobacterial extracellular polysaccharide not only helps cells to withstand desiccation, but aids the development of crust and soil structure. Understanding of crust structure and succession has proved important in planning reclamation programmes in semi-arid regions of China using cyanobacterial inocula. Details of the procedure are described, which sometimes includes techniques to minimize the effects of wind, such as the use a

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checker-board arrangement of protective straw to prevent the inocula from being blown away. Reclamation of semi-arid regions in other parts of the world will require similar understanding of the ecology of the cyanobacteria involved.

12.1 Introduction

It has long been known that cyanobacteria can have an important role in environments subject to periods of pronounced water stress, including many desert regions. Much of the earlier literature on deserts dealt with the visually more obvious organisms, such as the mixed colonies of *Calothrix desertica* and *Schizothrix atacamensis* in the Atacama Desert, Chile, described by Schwabe (1960). Early accounts of desert cyanobacteria were mostly taxonomic or floristic, but a study by Friedmann et al. (1967) in another hot desert, the Negev, Israel, helped to raise interest in ecological and physiological problems. Many more accounts of hot desert cyanobacteria followed within a few years. Accounts of cyanobacteria in Antarctic cold deserts also started to be published, but initially these adopted the taxonomic system of Francis Drouet (see Drouet 1981), as did other reports on the Atacama Desert, rendering the data of little value. However, several accounts by E.I. Friedmann on endolithic organisms in rocks of cold as well as hot deserts (Friedmann and Kibler 1980; Friedmann 1982) using mainstream taxonomic conventions stimulated widespread interest in the organisms. Not only did they establish that endolithic cyanobacteria and green algae are widespread in desert rocks, but studies on Antarctic dry deserts raised questions about the environmental limits for life (Friedmann and Ocampo-Friedmann 1984). This in turn led to assessing the possibility for life on Mars, whether organisms with similarities to cyanobacteria might even occur there, and the potential introduction of a cyanobacterium to provide a source of oxygen (Friedmann and Ocampo-Friedmann 1995). In recent years many studies have focussed once more on the Atacama Desert, because of the range of extreme environments there (McCay et al. 2003; Navarro-González et al. 2003) and the fact that it includes the driest non-polar location on Earth, with a mean of only 2.4 mm rainfall a year (Lacap et al. 2011). Wynn-Williams (2000) reviewed the literature up to that date for both hot and cold deserts, although with the emphasis on the latter. This review is 'reprinted' in the online material associated with the present book, so aspects covered by Wynn-Williams are reported only briefly here, while desert epiliths are mentioned in Chap. 10. The present chapter largely deals with biological crusts, considering only briefly those aspects covered in Chaps. 18 and 19. A short account of desert endoliths is given at the end.

Cyanobacteria in the biological crusts are the main primary producers in dry desert or related environments, though other microorganisms are plentiful in later stages of crust development. The cyanobacteria inhabiting desert crusts usually experience intense environmental stresses, including high daytime temperatures during the summer, low temperatures during the night in the winter, high radiation including visible and UV radiation, and frequent hydration dehydration cycles. Interest in desert microbial communities has increased greatly in recent years, because of concern about the spread of desert and semi-desert regions as a result of how land has been managed in regions with low rainfall and also because of climatic changes. The ways in which algae, and cyanobacteria in particular, can be used to minimize this problem are the particular interest of the authors. Because of the relevance of these studies to improving soil fertility in other regions, the chapter includes a few comments on cyanobacteria in soils.

12.2 Biological Crusts of Arid Regions

12.2.1 Characteristic Species

The relationship between distribution of soil cyanobacteria and environmental parameters has been studied over a long period and much of the earlier literature was summarized by Metting (1981) and the literature to 2000 by Whitton (2000; reprinted in online material). More detailed reviews of biological soil crusts can be found in the volume edited by Belnap and Lange (2001) and the article by Zhao et al. (2009). Based on these and other studies (Roger et al. 1987; Hong et al. 1992; Hu and Liu 2003b; Bhatnagar and Bhatnagar 2005; Chen et al. 2006a; Büdel and Veste 2008); Büdel et al. 2009), it can be concluded that water availability and factors influencing this, such as rainfall frequency of rainfall, and soil moisture content, together with clay and the amount of available phosphate, have the greatest influence on cyanobacterial biomass. However, estimates of diversity based on standard sampling methods still provide inconsistent results. To what extent this reflects weaknesses in sampling procedure and to what extent it reflects real differences in nature is still unclear.

Liu et al. (2001) concluded that higher fertility supports higher diversity, with the maximum number of species occurring in winter after long period of utilization of organic manure. However, a comparison by Zhang et al. (2011) of their results for Gurbantunggut Desert with other those from other deserts indicated that their study region shows the highest diversity of morphotypes, in spite of the fact that the soils are poor in nutrients. The biodiversity of an Antarctic soil decreased with increasing trophic status (Mataloni et al. 2000), while differences in soil chemical composition had no influence on cyanobacterial diversity in the Thar Desert of India (Bhatnagar et al. 2008). The situation will probably only become clear when there are detailed measurements of nutrient concentrations throughout the season for a range of deserts (see Sect. 12.2.3.6). Agricultural ploughing and weeding can

also affect the diversity. For instance, Liu et al. (1999) found fewer species on the surface of soil subsequent to ploughing (Liu et al. 1999). Kirkwood et al. (2008) concluded that a variable environment promoted diversification, and in particular selected for variation in ecotype more than phylotype.

The local distribution of desert algae shows obvious differences between different microhabitats, although the genera *Microcoleus*, *Schizothrix, Scytonema*, *Lyngbya*, *Nostoc* and *Phormidium* have been reported from the soils of many desert environments (Issa et al. 1999; Hu et al. 1999, 2003d, 2004). Among the species recorded as frequent in particular regions are *Microcoleus vaginatus*, *Nostoc punctiforme*, *N. paludosum* and *Tolypothrix distorta* in many deserts of North America (Flechtner 2007), *Microcoleus vaginatus*, *Scytonema javanicum*, *Schizothrix fragile, Phormidium tenue* and *Nostoc* in the hot deserts of North China, and *Microcoleus vaginatus* and *Phormidium murrayi* and closely related taxa in the hot desert of the Colorado Front Range, USA (Belnap and Gardner 1993; Freeman et al. 2009). *Microcoleus vaginatus* is the dominant species in most sandy soils (Belnap 1993; Hu et al. 2003b, d). In the cool part of the Qaidam Basin in China, *Phormidium foveolarum* and *Lyngbya diguetii* are most frequent in the Gobi region, whereas *Microcoleus vaginatus*, *Schizothrix undulata* and *Myxosarcina chroococcooides* occur more often in the eastern part, which is relatively rich in clay (Hong et al. 1992). Whereas filamentous forms are by far the most important among cyanobacteria, the next most important group of algal phototrophs are composed mainly of unicellular coccoid species (Zhao et al. 2009).

Cyanobacteria are sometimes also frequent in dry, but less extreme, regions than the deserts mentioned above. For instance, *Nostoc calcicola*, *Leptolyngbya nostocorum* and *Phormidium autumnale* were very abundant in fallow fields of (the then) Czechoslovakia (Lukešová 1993). In a comparison of four different types of highly cultivated land in N-E. Italy, a cornfield, vineyard, pasture and a field abandoned for 12 years, cyanobacteria were least frequent in the vineyard, being almost absent there (Zancan et al. 2006). In dry farmland of Fenqiu County, Xinxiang, *Dactylococcopsis*, *Fischerella* and *Synechococcus* only occurred in clay, loam and sandy soil, respectively (Liu et al. 2001). However, in the loess area of Lanzhou Wuquanshan and Lanzhou North Hill (Gansu Province) *Phormidium africanum* was the main dominant. In Czech forest soils, *Nostoc calcicola* showed the highest biomass (Lukešová 1993), whereas *Schizothrix telephoroides* dominated in South African savanna soils (Büdel et al. 2009).

12.2.2 Taxonomic Problems in Describing Crust Cyanobacteria

It can still be difficult to name cyanobacteria based on morphological and morphometric data, even if ultrastructure is considered (Vigna et al. 2001; Büdel 2005; Branco et al. 2009) and the problem tends to be especially difficult for dry soils, where the organisms often have fewer obvious features than in some other types of environment. New taxa are still being described (Flechtner et al. 2002). Analyses based only on microscopy generally underestimate the biodiversity of narrow filamentous cyanobacteria, so molecular approaches which may require isolation and culture are required (Schlesinger et al. 2003; Berard et al. 2005). Even molecular analyses can still under-represent signals for conspicuous heterocystous taxa with thick sheaths in biological soil crusts (Garcia-Pichel et al. 2001). However, an improved methodology involving both terminal restriction fragment length polymorphism (TRF or T-RFLP) analysis and 16S rDNA sequence analysis can provide accurate information about the composition and relative abundance of cyanobacterial types (Redfield et al. 2002). A combination of microscopy, DNA sequencing and amplified fragment length polymorphism (AFLP) proved useful in differentiating variation within *Nostoc commune* during extensive sampling across environmental gradients (Novis and Smissen 2006). Automated rRNA intergenic spacer analysis (ARISA) and 16S rRNA gene clone libraries were effective for the investigation of cyanobacterial diversity in a range of soil environments (Wood et al. 2008). Use of STRR1A (primer oligonucleotide 5′-CCARTCCCCARTCCCC-3′) was the most informative and highly effective in diversity analyses of *Anabaena* (Nayak et al. 2009). So, although species composition and frequency failed to show variation at the level of traditional methods due to the low resolution (Mataloni et al. 2000), there were conspicuous differences using two complementary molecular biological approaches (Mataloni and Tell 2002).

Molecular studies have been applied to *Microcoleus vaginatus*, which is a very common dominant in biological soil crusts throughout the world, including two of the studies mentioned above. The complete genome of one strain, *M. vaginatus* FGP-2, has been reported (Starkenburg et al. 2011). However, according to the results of16S rRNA gene and 16S-23S internal transcribed spacer region studies (Boyer et al. 2002), this is not a single species. Combined analysis of sequence and morphological data of 31 strains from desert soils in the USA revealed it to include two species, *M. vaginatus* and *M. steenstrupii*. The latter was suspected of representing several cryptic species, as it showed much greater genetic variability (16S similarities ranging from 91.5% to 99.4%) than the former (97.1–99.9%). The *M. steenstrupii* Boye-Petersen (1923: see Geitler 1932) came from thermal springs, whereas morphologically similar material has been reported not only from the deserts of the Boyer et al. (2002) study, but also hypersaline lakes in Afghanistan and soils in India (Komárek and Anagnostidis 2005). The genus has since been classified into two clades based on 16S rDNA phylogeny, with a new genus *Coleofasciculus* being

proposed (Siegesmund et al. 2008). (For simplicity, the name *Microcoleus* is retained in this chapter).

12.2.3 Environmental Factors

12.2.3.1 Light

The euphotic zones in biological crusts on sand are mostly only 1–2 mm thick due to strong light attenuation by the sand. In a study of cyanobacteria in desert soils in China, 96% could occur within the upper 1.0 mm of crust (Hu and Liu 2003a). Rates of gross photosynthesis and net respiration in the upper 1 to several mm of recently rewetted cyanobacterial crusts in two south.-eastern Utah crusts were sufficiently high to form marked oxygen microenvironments ranging from supersaturation to anoxia; localized pH values sometimes exceeded 10 (Garcia-Pichel and Belnap 1996). The ways in which the cyanobacteria adapt to the light environment include mechanisms for optimizing photosynthesis, protection from damage by visible and UV radiation and sometimes also movement of filaments away from the light. The different layers within soil crusts show differences in how they adapt to light intensity and light quality in the particular layer (Hu et al. 2003c).

Cyanobacteria in the crusts must be equipped with mechanisms to protect themselves against photoinhibition under high light intensities especially during dehydration, since photosystem II (PSII) in most cyanobacteria is highly susceptible, leading to rapid degradation of its core proteins, while subsequent repair requires *de novo* synthesis and reassembly (Niyogi 1999). It is essential for crust species that they can reverse metabolism rapidly and grow in the short periods when water is accessible and then hold back metabolic activity during dehydration. Existing proteins must be stabilized during dehydration to guarantee growth (Potts 2001). In a *Microcoleus* sp. dominated sand crust, over 50% of photosystem II (PSII) activity, assembled phycobilisomes, and photosystem I (PSI) antennae were detected within less than 5 min of rehydration, and energy transfer to PSII and PSI by the respective antennae was fully established within 10–20 min (Harel et al. 2004). Even at exceedingly high light intensities, photoinhibition of PSII occurs only to a limited extent and this is largely balanced by a fast rate of PSII repair. The semi-desert species *Nostoc flagelliforme* tolerates high levels of solar radiation even in the presence of UV (Gao and Ye 2007). In addition soil cyanobacteria can also protect against high light intensity and UV by scavenging reactive oxygen and free radicals (Wang et al. 2007a, 2008).

Other means of minimizing damage by high light intensities include the formation of molecules which dissipate some of the energy at potentially damaging wavelengths before it reaches sensitive regions of the cell. Terrestrial cyanobacteria show marked changes in pigment composition and content in response to visible and UV radiation (Chap. 19), with scytonemins, mycosporine-like amino acids (MAAs) and carotenoids content increasing with solar radiation (Bowker et al. 2002). Sheath-forming filamentous cyanobacteria are the major components in the upper millimetre of blackish-brown crusts, and the dominant species have high contents of carotenoid and MAAa with absorption peaks in the UV or at 507 nm (Tirkey and Adhikary 2005).

Another means of ensuring the population is maintained is for some trichomes to move downwards when light or UV radiation becomes strong. Most soil cyanobacteria can move downwards (Garcia-Pichel and Pringault 2001). Under weak light they can rapidly initiate photosynthesis when light intensity increases, although cyanobacteria deeper in a Baja California mat were shown to recover later than those near the surface (Fleming et al. 2007). There are many records of cyanobacteria at considerable depth in other soil types, such as *Nostoc* and *Leptolyngbya* at 16–18 cm in paddy soils in Osaka, Japan (Fujita and Nakahara (2006), but it is unclear how long such populations can persist in a healthy state.

12.2.3.2 Water

It is not yet possible to generalize on how different water regimes influence the type of crust and its vertical profile, because of the complexity of ways in which the regime can influence both soil formation and crust metabolism at a site. It seems likely that there are considerable differences between sites with rare, but intermittently high, rainfall, versus low rainfall at defined seasons and the almost total dependence on dew of some coastal deserts such as the Namib Desert, Namibia (Lalley and Viles 2005). In the Qubqi Desert of Inner Mongolia (40°21′N, 109°51′E), water vapour from the atmosphere accounted for 25–39% of the total water uptake by man-made crusts with *Microcoleus vaginatus* and *Scytonema javanicum* on a sandy soil (Lan et al. 2010a). This took place mainly by a water vapour adsorption mechanism. However, it did not occur immediately after inoculation of the surface with the cyanobacteria, but there was obvious increase by day 15 and the rate had become markedly enhanced by day 20. It was concluded that growth of cyanobacterial filaments and their associated EPS were the main factors increasing water uptake and content. The inoculated filaments were shown to be in direct contact with the sand and clay particles.

Whatever the particular type of crust, the ability to adapt to desiccation is the top requirement for the cyanobacteria; water content is more important than light, salinity and other factors for their distribution (Tsujimura et al. 1998). Research has shown that terrestrial cyanobacteria have a marked capacity to withstand removal of their cellular water (Potts 1999). The uronic acid of viscous extracellular polysaccharide (EPS) can regulate water retention properties by the transition of gel and sol state (Potts 1994) and is an integral component of cells subject to drying in their natural environment (Helm

et al. 2000; Chap. 18). The presence of a sheath and mucilage can help protect cells against physical desiccation, though not UV shock according to Gupta and Agrawal (2008), unless a UV-absorbing molecule such as scytonemin is immobilized on the EPS. However, Chen et al. (2009a) found that pretreatment of a culture of a desert strain of *Microcoleus vaginatus* with 100 mg L^{-1} EPS effectively eliminated reactive oxygen species and thus provided significant protection from DNA strand breakage and lipid peroxidation. Mager and Thomas (2010) reviewed the overall role of cyanobacterial EPS in soil crusts.

Despite the importance of the water content, a rapid change in it can sometimes be even more crucial for the organisms. The hair-like *Nostoc flagelliforme* can die if it is kept continuously in a very moist environment, because the water loss of physical evaporation is an active process influenced by inhibitors of transcription and translation (Shaw et al. 2003). A regular cycle of dehydration and rehydration is very common in terrestrial cyanobacteria and even indispensable in some cases. The response of *Microcoleus* to a fluctuating water content is especially important in developing the structure of soil biological crusts (Belnap et al. 2001). The bundles of *Microcoleus* trichomes surrounded by their extracellular sheath wind throughout the uppermost soil layers. When sufficiently moist, the trichomes glide out of their sheaths, show positive phototaxis and glide towards the soil surface. Upon drying, the trichomes leave the surface, and the exposed trichomes secrete new sheaths. Frequent changes in moisture content therefore result in copious EPS being dispersed throughout the soil.

Dehydration is, however, a stress process. In studies with desiccation-tolerant *Nostoc commune*, PSI and PSII were both deactivated during dehydration with the loss of photosynthesis; the evidence suggests that dissipation of the light energy absorbed by PSII prevents photoinhibition when subjected to strong light in a dehydrated state (Hirai et al. 2004; Fukuda et al. 2008). Rehydration is an induced process, with over 50% PSII activity and PSI antennae being detected within 5 min of rehydration (Harel et al. 2004). During this period the energy transducing reactions recover first, followed by an increase in ATP pool size (Tiwari and Tripathi 1998); the energy transfer to PSII and PSI can be fully established within 10–20 min of rehydration (Harel et al. 2004). After the recovery of the initial phase, a change in 77 K fluorescence emission spectra began (Qiu et al. 2004a). The recovery can be enhanced by the content of some ions (K, Mg, Ca, PO_4), because they enhance the probability of electron transfer beyond Q (A) and the recovery of electron transport flux per PSII reaction centre (Qiu et al. 2004b). From an ecological viewpoint the speed of water uptake depends upon the duration of desiccation (Shaw et al. 2003) and the type of habitat (Tirkey and Adhikary 2005).

Many factors interact in the natural environment and their influence can differ between different desiccation-tolerant organisms. For instance, Fleming and Castenholz (2007) reported that increased UVA radiation led to a more concentrated scytonemin screen in *Nostoc punctiforme* PCC 73102 and *Chroococcidiopsis* CCMEE 5056, when subjected to periodic desiccation, in comparison with cells maintained fully hydrated. A more concentrated scytonemin screen would reduce the amount of UVR damage accrued when cells are desiccated and metabolically inactive. This might allow the cyanobacteria to allocate more energy during rehydration to systems other than UVR damage repair, which would facilitate recovery. The scytonemin screen is extremely stable, remaining largely intact in the sheaths of desiccated *N. punctiforme* even when continuously exposed to UVA radiation for 2 months. In contrast, scytonemin synthesis in *Chroococcidiopsis* CCMEE 246, a strain producing scytonemin constitutively under low visible light and no UVA was partially inhibited by periodic desiccation.

During the recovery process, extracellular polysaccharides have a very important role in maintaining cell morphology (Chen et al. 2006b). Part of the reason for this is that the extracellular polysaccharide can generate superoxide radicals when rehydration occurs, and the Fe-SOD can counter the effects of oxidative stress (Shirkey et al. 2000). At the same time external carbonic anhydrase can also be activated by rehydration (Ye et al. 2008) and these activations may lead to time-dependent changes in structure and ultrastructure and fluctuations in the composition of the transcriptome. More genes, especially those involved in DNA repair, protein folding, NAD synthesis, nitrogen depletion and CO_2 limitation, might be specifically down-regulated or up-regulated (Higo et al. 2007).

The gene *sigJ* is now considered as a fundamental and conducive gene for desiccation tolerance in cyanobacteria, as it up-regulates a large number of genes relating to polysaccharide biosynthesis (Yoshimura et al. 2007). Although *Nostoc commune* can survive several decades of desiccation (probably many more), genomic DNA is still covalently modified during this period, which leads to the loss of supercoiling aggregation, transformation and transfection efficiency. Thus, this response is different from that of *Deinococcus radiodurans* to ionizing radiation (Shirkey et al. 2003). Scondly, acidic WspA may be an important protein in desiccation adaptation, playing a role by modulating the structure and function of the three-dimensional extracellular matrix, or by influencing the distribution and transportation of mycosporine and scytonemin (Wright et al. 2005), as indicated by the influence of drought on scytonemin production in at least some cases (Fleming and Castenholz 2007). Finally, the gene cluster including *treZ*, *treY* and *treH* is very important. This cluster can increase the rate of trehalose production under water-stress conditions (Yoshida and Sakamoto 2009), while trehalose can stabilize membranes (Potts 1994), so the accumulation of trehalose minimize damage to DNA during drought periods (Shirkey

et al. 2003). Trehalose was also one of the substances formed as part of the adaptation by cyanobacteria in the Antarctic fellfields to low temperatures (Arnold et al. 2003).

12.2.3.3 Temperature

Although extreme temperatures may be expected to be detrimental to phototrophs, cyanobacteria still dominate sandy surfaces at 40–52°C and −9°C (Arnold et al. 2003). The soil surface temperature where *Nostoc flagelliforme* grows can exceed 65°C. In the study by Chen et al. (2011) on crust development by this species (Sect. 12.5.2), the authors compared the effects of drying material for 2 h at 60°C with a control on photosynthesis and respiration rates in liquid culture. There was a slight reduction in both activities for the material which had been heated, but nevertheless the rates were still high.

In comparison with most aquatic algae, soil cyanobacteria are much more tolerant to temperature changes and to interactions between temperature and humidity; it is important to consider the response of natural populations to temperature stress together with their response to water stress. Moist cyanobacteria may differ in their sensitivity to heat (Gupta and Agrawal 2006), while the same strain can show obvious differences between different growth stages (Agrawal and Singh 2000). Akinetes usually have a higher tolerance than other stages. Although many akinetes in the study by Agrawal and Singh germinated at 35°C, their formation was markedly suppressed at this temperature, and at no temperature did heat shock promote akinete formation or germination. It was concluded that both wet and dried akinetes tolerated dryness, but not frost. However, such studies must be considered in relation to the particular environment from where the organisms had been sampled.

Most soil cyanobacteria survive equally well or slightly better when exposed to the air on a moist soil surface than when suspended in liquid medium. Desiccation-sensitive algae survive high atmospheric humidity better than desiccation-resistant algae, indicating the importance of atmospheric temperature and relative humidity for a particular species (Gupta and Agrawal 2008). Agrawal and Pal (2003) compared the effects of water stress using agar medium or exposure to NaCl solution on five cyanobacteria and two green algae for various lengths of time. Somewhat surprizingly, two of the three most sensitive organisms were dried mucilaginous small-celled cyanobacteria. Nevertheless the authors concluded that the tolerance of micro-algal forms to water, heat or UV stress depended primarily upon cell-wall characteristics or cellular osmotic properties rather than the original habitat, morphology or whether prokaryotic or eukaryotic.

As part of a programme on the possible effects of climate change, a detailed study was made on the influence of environmental factors on carbon fixation by *Nostoc commune* in Victoria Land, continental Antarctica, where the organism is a very conspicuous terrestrial primary producer (Novis et al. 2007). The study, which involved field and laboratory measurements, together with results from previous years, led to a model describing the process over 1 year. Desiccated *N. commune* mats with a water content ≤30% saturation, showed such a variable rate of net C fixation between replicates that the data could not be modelled. However, for colonies at >30% saturation, the rates of net C fixation and dark respiration depended strongly on irradiance and temperature. Annual net C fixation was 14.5–21.0 g C fixed m^{-2} *Nostoc* mat, depending on the year. Estimates for different seasons correlated with thermal time (accumulated hours above 0°C during the year) rather than irradiance, in contrast to phototroph communities in local (Antarctic) lacustrine environments, where irradiance is the main driver of primary productivity. The relationship between thermal time and net annual C fixation by *N. commune* was strongly linear. *N. commune* appears to compromise between an ability to capitalize on short periods of higher temperature and efficient utilization of lower irradiance at low temperature.

12.2.3.4 Salinity

Although *Dunaliella* spp. are the most abundant phototrophs at the most consistently hypersaline sites in the Salt Plains area of Oklahoma, USA, cyanobacteria have the highest biomass at sites which experience greater fluctuations of salinity (Kirkwood and Henley 2006). They show high diversity in such environments, this diversity spanning a number of cyanobacterial lineages (Kirkwood et al. 2008). Salt stress is accompanied by water stress and can inhibit growth, decrease the Fv/Fm ratio, photosynthetic yield of a mat, slow down photosynthetic recovery, and lead to oxidative damage and lipid peroxidation (Chen et al. 2003b; Fleming et al. 2007; Tang et al. 2007a). Other responses include increases in trehalose and sucrose phosphorylase activation (Page-Sharp et al. 1999) and increases in exopolysaccharides and intracellular sucrose to maintain the cellular osmotic equilibrium between the intra- and extracellular environment (Chen et al. 2003a, b, 2006c). Production of antioxidative enzymes (SOD and CAT) helps counteract oxidative damage (Tang et al. 2007a). Cell morphology may also have an influence, as *Nostoc* with spherical cells showed a higher tolerance to water stress and salinity than *Nostoc* with cells of other shape; the former was able to rectify problematic soil better than the latter (Obana et al. 2007).

As colonizers of desert soil, cyanobacteria often encounter both drought and salinity. Growth and photosynthetic activity of a *Microcoleus vaginatus* crust was significantly inhibited by the double stress; the inhibitory effect increased with increasing intensity of stress and treatment time (Lan et al. 2010b). In contrast to salt stress, drought completely stopped crust metabolic activity, so the crust biomass was conserved at a higher level, which meant that drought alone can provide the crust some protection.

12.2.3.5 Carbon Dioxide

Terrestrial cyanobacteria can directly use CO_2 and HCO_3^{-1} (when hydrated) and an increased CO_2 concentration can enhance diurnal photosynthesis and raise the daily photosynthetic production. However, air-grown mats of *Nostoc flagelliforme* showed higher photosynthetic affinity for CO_2 than high-CO_2-grown ones (Qiu and Gao 2002). Soil CO_2 flux and the influence of soil phototrophs, including cyanobacteria, was studied in some detail at three sites above 3,600 m in the Colorado Front Range, USA (Freeman et al. 2009). The authors grouped such high-elevation areas with other soil-dominated ecosystems – deserts, polar regions and zones of glacial retreat, all of which have often been described as 'barren', despite their potential to host photoautotrophic microbial communities. A combination of soil CO_2 flux measurements and molecular techniques was used to characterize the types of soil phototroph and measure rates of CO_2 uptake. Soil CO_2 flux data from two different years indicated that light-driven CO_2 uptake occurred on most dates. A diverse community of cyanobacteria, "chloroflexi" and eukaryotic algae was present in the top 2 cm, whereas they were nearly absent in deeper (2–4 cm) soils. The cyanobacterial communities were composed of lineages most closely related to *Microcoleus vaginatus* and *Phormidium murrayi*. During the light hours of the 2007 snow-free measurement period, CO_2 uptake was estimated to be 23.7 g C m^{-2} $season^{-1}$. The authors concluded that photoautotrophic microbial communities play an important role in the biogeochemical cycling of subnival zone soil. (No studies were included to assess the possible contribution of photoheterotrophy.)

Several methods have been used for measuring CO_2 uptake by terrestrial cyanobacteria, but studies by one of the authors (KG) has found that use of an assimilation chamber to hold the sample and measurements of CO_2 concentrations in the inlet and outlet flows with an infrared gas analysis is the most straightforward. Practical details include replacing the cover of the assimilation chamber with a quartz cover and the need to maintain a constant CO_2 concentration in the inlet air flow. This can be achieved with an air bag, which also permits rapid increases in the CO_2 concentration by injecting pure CO_2 into the bag. Reduction of the CO_2 concentration can be achieved by pumping the ambient air though a soda lime column. If required, the open system can be converted to a closed one by circulating the gas.

12.2.3.6 Nutrients

In the Shapotou area of the south-east part of the Tengger Desert, the maximum biovolume of algae (ca. 95% biomass from cyanobacteria) was exhibited in August with the highest precipitation; the minimum value in February with the lowest air temperature (Hu and Liu 2003c). Six microclimate factors (wind speed, air and surface temperature, evaporation, precipitation, humidity) and 27 soil microenvironment parameters (total N, P, K, rapidly available N,P, K, C/N, organic matter, moisture, pH, electric conductivity, Ca^{2+}, Mg^{2+}, SO_4^{2+}, Cl^-, Mn, V, Zn, Cu, Fe, Co, coarse sand grains, fine sand grains, coarse silt, fine silt and coarse clay particles) associated with biovolume were considered. Stepwise multiple regression indicated that biovolume was positively correlated with the amount of local precipitation, total K_2O, soil hydrolyzable N, Fe^{3+} and coarse silt, while negatively correlated with pH, organic matter, Cu and Zn. It was also affected by the trace element Co.

There have been only a few reports on the role of nutrients in determining species composition or succession in crusts, but, judging by the frequency of heterocystous cyanobacteria, nitrogen fixation is an important N source. It therefore seems probable that P and perhaps Fe are the elements whose availability are the most likely to influence cyanobacterial growth. Total P and available P were reported to be the main chemical factors influencing the diversity of cyanobacterial and eukaryotic algal morphotypes soil samples from sand dunes in the Gurbantunggut Desert (Zhang et al. 2011). Mg content was also a factor influencing microalgal biomass and the authors suggested that the contents of P and Mg, soil texture and soil moisture were the main factors influencing distribution in this region. However, in experiments on rewetting dried *Nostoc flagelliforme* collected from Siziwangqi, Inner Mongolia, addition of K^+ had the greatest effect on recovery of photosynthetic activity, whereas Fe^{3+}, Mg, Na, NO_3, PO_4-P and Cl showed little effect (Qiu and Gao 1999). In the case of the cyanobacteria used for inoculation of semi-arid soils, such as the studies by the Liu and Hu group, the P-rich medium BG11 was used to prepare the material. The organisms are therefore probably in a P-rich state when reaching the soil and the cells can be expected to divide several times before becoming entirely dependent on ambient phosphate in the soil. No studies have been reported on the ability to use organic phosphates, but most of the cyanobacterial genera are ones which typically show high surface phosphatase activity in other environments (Whitton et al. 2005). The hypothesis is suggested that surface phosphatase activity per unit biomass is likely to increase during successional stages of crust formation.

12.2.3.7 Wind and Burial by Sand

Many desert regions are subject to strong wind action and hence cyanobacterial species and strains may be expected to dispersed widely and hence organisms best suited for growth at any particular site are likely to arrive quite often. The use of artificial inoculation (Sect. 12.4.1) in semi-arid regions merely shifts the ratio of cells to soil. The possibility that such organisms in desert dust in southern Iraq might have been sufficiently dense to act as a factor causing ALS (amylotrophic lateral sclerosis) disease in troops involved in the 1990–1991 Gulf War was raised by Cox et al. (2009).

The role of biological crusts in stabilizing soil against wind action was first described by Booth (1941) and subsequently there have been many comments on the importance of the filamentous microorganisms in the crusts (e.g. Issa et al. 1999; Tirkey and Adhikary 2005). This is especially important in sandy soils, because of their lower resistance to wind erosion than other types of soil (Zhang et al. 2006). These authors showed that the extent of erosion of biological soil crusts in the Gurbantunggut Desert, northern Xinjiang, Northwestern China, paralleled the extent of which the crusts had been disturbed. Wind tunnel comparisons showed that the maximum velocity tested, 25 $m\,s^{-1}$, caused no erosion of the surface of a crust with 100% cover, while the highest disturbance occurred without crusts. The wind erosion rate of sandy soil with 0% crust cover was 46, 21 and 17 times that of soil with 90% crust cover at wind velocities of 18, 22 and 25 $m\,s^{-1}$, respectively. The authors emphasized the importance of minimizing crust disturbance in desert regions. In addition to reducing trampling, strategies should be developed to manage livestock and oil exploration in order to avoid concentrated zones of impact. In a less extreme semi-arid region of southern Australia, biological soil crusts were shown to recover quite rapidly after livestock had been removed (Read et al. 2011). The authors compared their estimate of 20 years for recovery with the results of three other studies on recovery from livestock damage in desert regions of other parts of the world. The recovery rates were surprizingly similar, in spite of wide differences in annual rainfall (390 mm in Australia and 95–230 mm for the others). Read et al. concluded that mosses were the dominants colonizers in their region, but no studies were included on possible changes in cyanobacterial populations immediately after the livestock had been removed. However, it seems clear that recovery from livestock damage is quite different from colonization of a bare surface.

As cyanobacteria are usually the main component of the crusts, their ability to withstand wind force is important. Wind force is a stress factor and can decrease PSII activity and electron transport rate. In the case of *M. vaginatus* increases were recorded in proline, total soluble sugar, reducing sugar, extracellular sugar and protein contents (Xu et al. 2010). However, the fact that natural crusts are a mixture of several species makes it difficult to quantify the contribution by individual species, especially to cohesion. Several authors have proposed methods to deal with the problem (Liu et al. 1994; McKenna-Neuman et al. 1996). Hu et al. (2002a) isolated the dominants (*Microcoleus vaginatus*, *Phormidium tenue*, *Scytonema javanicum* and *Nostoc* sp.) from crusts in the Tengger Desert (37°32′N, 105°02′E), NingXia Autonomous Region, and measured their cohesion in stabilizing shifting sand. Biomass, species, community composition, niche within the crust, crust thickness, growth phase, soil moisture and dust accretion could all could increase the strength of cohesion. The threshold friction velocity (TFV) was increased significantly by all the species tested, but the increase was much greater with filamentous species. Among the four species *Microcoleus vaginatus* was the strongest, though *Phormidium tenue* was also strong indoors, but not outdoors. Thick crusts were less easily eroded than thin crusts, but biomass was more effective than thickness.

Cyanobacterial crusts throughout the world often encounter burial by sand during the course of their development at lower sites. In a greenhouse experiment Wang et al. (2007b) found that EPS content and *Fv/Fm* decreased correspondingly with the increase in the burial time and burial depth; however, the degradation of chlorophyll *a* commenced only at 20 or 30 burial days. This suggests that burial by sand is not an important stress, because, although cyanobacterial crusts may often be covered by sand, but they are seldom buried for long periods in naturally windy environments. Growth and/or motility give the organisms some ability to move upwards and this is probably an important part of the process of crust formation in such environments.

12.3 Development and Succession of Biological Soil Crusts

12.3.1 Crust Structure

The fact that the blackish-brown colour of many biological soil crusts is due to filamentous sheath-forming cyanobacteria has been shown by many authors (e.g. Tirkey and Adhikary 2005). The results indicate that microbiotic cover is an important determinant of sand fixation in the Gurbantunggut Desert, northern part of Xinjiang, Northwestern China. The crusts consist of an intricate network of filamentous cyanobacteria and EPS, which in sandy regions binds and entraps the sand grains, and conglutinates fine particles (Zhang et al. 2006). The crusts resemble other microbial mats and stromatolites in that there is a vertical stratification of different functional groups of microorganisms (Davey and Clarke 1992; Garcia-Pichel and Belnap 1996; Stal 2000; Hu et al. 2003c). The crusts are often distinctly laminated into an uppermost, partially inorganic, layer (*ca* 0.0–0.02 mm), a middle phototroph-dense layer (*ca* 0.02–1.0 mm) and the lower phototroph-sparse layer (below ca 1.0 mm) (Davey and Clarke 1992; Hu et al. 2003c). Cyanobacteria are distributed throughout the whole crust profile, and trichomes can move in response to light and water, as described for *Microcoleus* in Sect. 12.2.3.2 (see also Garcia-Pichel and Pringault 2001; Pringault and Garcia-Pichel 2004). Nevertheless, *Scytonema*, *Nostoc* and *Calothrix* are typically organisms of the uppermost layer (Hu et al. 2003c; Rosentreter and Belnap 2003), *Microcoleus vaginatus* of the middle layer, and *Phormidium* and *Lyngbya* of the deep layer (Hu

et al. 2003c). An experimental study (Wu et al. 2011) on crusts from a non-irrigated area of the Tengger Desert showed that 60% of the total algal biomass (presumably mostly cyanobacteria) occurred in the top 1 mm of the soil profile in algal soil crusts, but 80% in lichen soil crusts. The authors suggested that most of the algae in the latter were symbiotic.

A study of the micromorphology of the crusts under plane polarized light found no soil aggregates, apart from some micro-aggregates (Hu et al. 2003c). However, some interlocked micro-beddings exist in the profiles of older crusts, which are composed of fine clay particles formed by the deposition of fine silt; the shapes of these micro-beddings were thought to be related to the direction of water movement on the soil surface, and were the main factor reducting infiltration of the soil (Hu et al. 2004).

12.3.2 Crust Development

Once biological crusts start to develop, the sand surface becomes stabilized and the uppermost several millimetres of topsoil undergoes various changes. *Nostoc*, *Scytonema, Calothrix*, *Microcoleus* and hyphae within the soil profile were found to be nearer to the surface in older crust (Hu et al. 2003c; Rosentreter and Belnap 2003). It was suggested this aided the transformation from algal crusts to the lichen crusts of later stages (Hu et al. 2003c). There are also differences in the relative proportions of biomass. When lichens and mosses accounted for less than 41.5% of the crust surface, algal biovolume became higher in the older crusts, but the opposite applied when they covered more than 70% of it (Hu and Liu 2003a).

During development of the crusts there are many changes in physical and chemical properties. The amount of dew formation increases with the development level of the microbiotic crusts (Liu et al. 2006; Rao et al. 2009a), as do the concentrations of organic matter and nutrients, conductivity, porosity, silt, clay, secondary minerals, the bulk density and the number of microlayers of the crusts (Hu and Liu 2003c; Guo et al. 2008). CO_2 exchange rates have also been used as an indicator of the state of the development level (Zaady et al. 2000). In a study by Yan-Gui et al. (2011), the net photosynthetic rate was significantly higher at 51-year old restoration site in the Tengger Desert than a 15-year old site (1.57 versus 0.92 μmol m^{-2} s^{-1}). Accumulated carbon fixation also increased with restoration time, but with a slower phase initially and then higher one after 43–51 years of restoration. The accumulated carbon fixation was correlated with soil organic carbon content. Later successional crusts have been shown to have not only greater photosynthetic activity, but also greater nitrogenase activity and N_2 fixation, and daily Fv/Fm (Housman et al. 2006; Rao et al. 2009b).

Other biological phenomena have been reported with the progress of the successional stages. Desert protozoa were shown to be adapted to a specific temperature and precipitation regime during crust development (Darby et al. 2006), their numbers increasing during succession from *Microcoleus* to lichen to bryophyte crusts (Bamforth 2008). The mechanism of this aboveground – belowground link is probably due to the increase in food, habitat, nutrient inputs, moisture retention and environmental stability provided by the biological soil crusts. Isopods have an important role in nutrient cycling (Shachak et al. 1976). Nematode status has been used as a biological indicator of soil condition, with successionally more mature communities (abundance, richness and diversity) beneath well-developed, late-stage crusts than beneath immature, early-stage crusts (Darby et al. 2007). The desert snail *Sphincterochila zonata* was on a loess plain in the Negev Desert an important role in grazing the surface algal community, in spite of the fact that it was only active on 8–27 winter days annually (Shachak and Steinberger 1980). Other reports concern organisms higher in the food web, such as the distribution of lizards (Zaady and Bouskila 2002). CO_2 exchange rates have also been used as an indicator of the state of the development level (Zaady et al. 2000). Later successional crusts had greater photosynthesis, nitrogenase activity, higher daily C and N fixation, and daily Fv/Fm (Housman et al. 2006; Rao et al. 2009b). It is clear that the formation of biological soil crusts can speed up development of the soil ecosystem (Chen et al. 2002).

By comparison with the methods of mineralogy and agrology, biomethods (such as algal distribution and biovolume) are more precise in judging algal crust development because the changes in minerals and soil are very slow (Hu et al. 2003c). The results of molecular fingerprinting (Garcia-Pichel et al. 2001; Redfield et al. 2002) also indicate that a particular microbial community corresponds to the degree of crust successional maturity (Gundlapally and Garcia-Pichel 2006). With respect to diazotrophs, the abundance of *nifH* sequences and nitrogenase activity were both higher in mature crusts than in poorly developed ones (Omoregie et al. 2004). Yeager et al. (2004) also demonstrated a transition from a poorly developed crust to mature crusts harboring a greater percentage of *Nostoc* and *Scytonema* spp.

With respect to the developmental stage, Li et al. (2002) divided crust development into the three stages, a non-biological crust, crusts enriched with mosses, and crusts dominated by abundant algae, mosses and liverworts. However, this classification merely reflected the distribution pattern in a natural environment rather than the biological development process. Development has usually been considered in three stages, cyanobacteria-dominated, lichen-dominated and moss-dominated ones (Rivera-Aguilar et al. 2006). This classification fits neatly with the evolutionary position of the

three types of dominant and is consistent with the typical process of biological development. However, it can be difficult to differentiate crusts at the middle and late stages, because they are often a mixture. In studies by researchers at Wuhan records are based on the dominant coverage and crusts with similar cover of one of the dominants are treated as the same transition stage (Lan et al. 2011b).

Particular interest has been given to the development of, and crust formation by, *Nostoc flagelliforme* because of its economic value (Sect. 12.3.3 and Chap. 26). Growth has been studied on filter paper, various soil types (Hu et al. 1987), transparent plastic sheets (Gao and Yu 2000) and sand (Chen et al. 2009b, 2011); apart from the plastic, all the tests were conducted in petri dishes. Comparison of coarse (between 5 and 14 Tyler mesh) and fine sand (<32 Tyler mesh) showed that growth was faster with the fine sand. Electron microscopy showed that cells of the filaments adhered closely to the sand surface. The biological crust was a multilayer network formed by EPS mingled with filaments intertwined around each other.

12.3.3 Succession

Studies of succession on newly exposed surfaces where conditions are unfavourable for rapid invasion by rooted plants have provided many examples where cyanobacteria have an important role during the early stages. In the case of gypsum rocks in S-E. Spain succession started with domination by cyanobacteria, which was then followed by green algae (Dana and Mota 2006). In the Tengger Desert succession fell into three stages, with *Microcoleus vaginatus*, *Scytonema javanicum* and *Microcoleus vaginatus* dominating, respectively, at the three different stages (Hu et al. 2003d). *M. vaginatus* was the first colonizer on all microenvironments and had a marked success over other algae. The process was affected by water content, nutrient concentrations and extent of shading, with shortage of water being the most important Under irrigation conditions and where the slope was less than 45°, a succession of dominants could occur within the first 1–2 months, by which time nutrient levels were low and *S. javanicum* took over. Unconsolidated sand on a sandhill in the Laarder Wasmeer, an inland dune area in The Netherlands, was colonized initially by *Oscillatoria* spp., this being followed in turn by *Klebsormidium*, *Synechococcus* and *Zygogonium* (Pluis 1994). The algal crust seldom reached maturity on the dunes due to wind action, but the crust was more stable on flat areas. However, when the green alga *Zygogonium* did form a pronounced cover on slopes, the crust became water-repellent when dry, leading to higher surface run-off during rainfall. This region merits further study, because the importance of unsheathed cyanobacteria at the first stage of succession contrasts with reports from most other areas.

The usar soils (alfisol, solonetz, alkaline) of India have been the subject of many studies on colonization following the pioneer research of R.N. Singh (1950) and his students. In a study by Pandey et al. (2005), the obvious growths of cyanobacteria after the first monsoon rainfall appeared in the order: *Microcoleus* sp., *Calothrix brevissima*, *Scytonema* sp., *Cylindrospermum licheniforme*, *C. fertilissima*, *Nostoc calcicola*, *Nostoc punctiforme*, *Aphanothece parietina*, *Nostoc commune*, *Aulosira fertilissima*, *Phormidium* and *Oscillatoria.* Among these, *Nostoc calcicola* was the dominant. It was suggested that differences in colonization were related to local climate, the importance of air-borne inocula and other factors concerning soil and biology.

The next stage in succession typically leads from the algal crusts to crusts dominated by lichens and/or mosses. Hu and Liu (2003b, c) found that the abundance of cyanobacteria decreased gradually during succession, especially that of *Scytonema javanicum*, whereas biodiversity increased gradually. Biomass increased at an early stage, but then decreased again. Studies on crusts in South Africa showed that crust thickness and chlorophyll content increased significantly as the early successional types progressed to late successional types (Büdel et al. 2009). However, the speed of natural succession was so slow that the community-building species were still the first dominant species after 42 years, although the degrees of dominance had decreased slightly. Hu and Liu (2003a) found that the speed of succession and trends at sites in the Tengger Desert were affected by water, vegetation coverage, terrain, time and soil physical and chemical properties, especially the Mn content in the soil, which appeared to have a threshold effect.

In a study (Büdel et al. 2009) of drylands in South Africa, the main factors influencing diversity and distribution patterns of biological soil crusts were rain frequency and the duration of dry periods rather than total precipitation. Silt and clay had a significant positive correlation with the number of crust types, and cyanobacterial diversity was significantly higher in the winter rain zone than the summer rain zone. This led the authors to suggest that fine grain-size could promote crust succession and their biomass content. However, Li et al. (2002) thought the most significant driving factor in the development of microbiotic crusts in the Tengger Desert was the spatial variability of rainfall infiltration depth (Li et al. 2002). Some insight as to how such variability can occur was provided by Gao et al. (2010) in a study on Mu Us Sandland soils. Under high water conditions, soil water in the surface soil layer (0–10 cm) was higher in soils with biological surface crusts than those without them, while the opposite was true in the deep soil layer (30–55 cm). However, under low water conditions, surface water was lower in the presence of crusts than in their absence.

Many studies have been reported on the influence of soil crusts on the next stage in succession, the colonization and

subsequent growth of vascular plants. However, it is still often unclear what cyanobacteria contribute at this stage (Büdel 2005). The importance of nitrogen fixation in soil crusts is often stressed and much of this is likely to be contributed by cyanobacteria, whether free-living or a lichen symbiont. In a study (Lesica and Shelly 1992) of the effect of biological crusts on growth of the rare endemic *Arabis fecunda* in S-W. Montana, USA, the crusts were the pinnacle type, which has a highly dissected surface. At least three of the four phototrophs listed (*Microcoleus vaginatus*, *Nostoc commune*, *N. muscorum*, *N. punctiforme*) and several of the lichens are N_2-fixers. The presence of a crust favoured survival of older plants of *Arabis*, but not its recruitment.

12.4 Applications

12.4.1 Desert Reclamation

Cyanobacteria as the pioneers of biological soil crust formation can have a range of effects. These are ability to stabilize the topsoil (Hu et al. 2004), increase and maintain soil fertility (Tirkey and Adhikary 2005; Yan-Gie et al. 2011), adsorb and capture dust (Hu et al. 2002b), enhance topsoil moisture (Garcia-Pichel and Pringault 2001; George et al. 2003) and facilitate soil reclamation (Liu et al. 2001; Pankratova 2006; Obana et al. 2007). This all supports the suggestion that inoculation of cyanobacteria is a suitable biotechnological approach for reclaiming desert soils (Acea et al. 2003). However, until recently only natural soil crusts, discrete fragments and a slurry of mixed cyanobacteria and lichen have been tried in an experimental scale (Belnap 1993; Hu et al. 2002b) and on a degraded soil (Maestre et al. 2006). Several authors therefore concluded that this was underexploited opportunity for restoring soil ecosystems (Liu et al. 2001; Bowker 2007).

The practical application of cyanobacterial inoculation on the shifting sand dunes of the Qubqi (Hobq) Desert of Inner Mongolia (Fig. 12.1) commenced in 2001 (Chen et al. 2006a; Tang et al. 2007b; Xie et al. 2007). By 2005, at the end of this particular programme, the inoculated area had reached ca. 0.033 km^2 and the coverage of biological crusts was over 70%. During this period spraying of the inoculum was carried out by workers.

Since 2007 more than 4,880 m^2 race-ponds for algal culture have been built and about 25 km^2 sand dunes had been inoculated (up to 2010) in the Qubqi, Ulan Buh and Mu Us Deserts. By October 2010 another 15 km^2 had been inoculated in the Horqin (Keerqin) Sand Land and the Hulun Buir Sand Land. The results so far indicate that the crustal cover can reach 40–50% in the first year, 60–70% in the second year and more than 70% by the third year, except for sites subject to strong winds. Inoculation is accomplished using a truck (tanker)

Fig. 12.1 The flat, shifting sand region of the Qubqi Desert, Dalateqi, Inner Mongolia, with sparse short shrubs (*Parthenocissus tricuspidata*) and the grass *Elymus dahuricus* in August 2007. The area will be sprayed with a suspension of 50% *Microcoleus vaginatus*, 20% *Scytonema javanicum* and 30% *Phormidium tenue*

(Fig. 12.2) modified to provide the spray (Figs. 12.3 and 12.4). The technology combines cyanobacterial inoculation with planting shrubs (Fig. 12.5), branches or straw, the latter two being arranged in a checker-board pattern (Figs. 12.6 and 12.7). This procedure has been applied mainly to the sides of railways and highways passing through arid land and to the margins of oases and farming-pastoral zones in north China. The principle of the technology is to inoculate different cyanobacterial communities onto an unstable sandy surface taking into account the local climate, soil texture, vegetation cover and characteristics of the terrain. In lower land among sand dunes and flat patches inoculation by cyanobacteria alone has usually proved sufficient, but on steep slopes (Fig. 12.8) this needs to be combined with the branch (or straw) checker-board technique, or shrubs planted to reduce the slope before inoculation.

The short-term aim is to speed up the formation of biological soil crusts to stabilize the shifting sand surface; while the long-term or final objective is to facilitate the restoration of the soil ecosystem as a whole, including the establishment of other cryptogamic plants (eukaryotic algae, fungi, lichens, mosses), vascular plants (grass and shrub) and heterotrophs (protozoa etc.), and the enhancement of soil fertility and the reclamation of soil structure.

What have proved to be the ideal inocula for these Chinese semi-desert regions are approximately 60% *Microcoleus vaginatus*, 20% *Scytonema javanicum* and 20% *Phormidium tenue* (*Leptolyngbya tenuis*). The principle in selecting materials has been to use inocula simulating nature. It is useful to consider the roles of these three species. In more than 90% natural crusts, *Microcoleus vaginatus* contributes the most cohesion in stabilizing a sandy surface; it is the first dominant

Fig. 12.2 The vehicle carrying water for sites adjacent to the road in the eastern part of the Qubqi Desert, Jianchai, Inner Mongolia

Fig. 12.4 Spraying water up to c 100 m distance onto an area already inoculated with algal suspension. Qubqi Desert, Jianchai, Inner Mongolia

Fig. 12.3 Truck equipped with an improved sprinkler head spraying an algal suspension up to a distance of 50 m. Qubqi (Hobq) Desert, Dalateqi, Inner Mongolia, in August 2007

Fig. 12.5 The space between two rows of *Salix psammophila* planted in an area of shifting sand to be sprayed with algal suspension. Qubqi (Hobq) Desert, Dalateqi, Inner Mongolia, in August 2008

and often forms the middle layer of an algal crust. *Phormidium tenue* often forms the deep layer within the crust; although it is able to stabilize the sand surface quickly because it provides the strongest cohesion; however, it is unable to survive on most surfaces due to its weak tolerance of radiation. In the case of *Scytonema javanicum,* its ability to provide cohesion in stabilizing the sand surface is not so strong as the other two species, it is the strongest against radiation damage (both high light and UV). If often occurs in the most uppermost layer, thus protecting the species below. The combination of the three provides strong resistance to damage by wind and radiation. As all the strains were taken from local natural crusts, nothing has been done that might influence the genetic composition of natural communities.

The group involved with the project at Wuhan considered that this approach was indispensable and should be at the forefront of research in areas prone to desertification. Before this the most promising biological technique to control desertification had been limited to the planting of vascular plants in fields (Kang 1999). The trees needed several years to grow and, even if they had survived and grown, the sandy surface remained unstable due to the shrub or tree cover being less than 30%. The inoculation technology solves this problem and suits the ecological characteristics of the drought region and represents the direction in which desertification control should be achieved (Yang et al. 2001). The main difficulty is the high cost, because the mass culture of cyanobacteria is expensive and long distance trans-

Fig. 12.6 Because of the strong winds in the region, a checker-board pattern of areas is marked out with small branches before the surface is inoculated with algae. Qubqi Desert, Dengkou, Inner Mongolia, in August 2008

Fig. 12.8 The process of placing the straw checker-board on slopes before inoculation. Qubqi Desert, Dalateqi, Inner Mongolia, August 2008

Fig. 12.7 The high dunes of the Ulan Buh Desert are covered with a checker-board of branches before the surface is inoculated to reduce the effect of strong winds. Dengkou, Innner Mongolia, in March 2007

port of fresh material is not easy; unfortunately, the activity of dry powder is always much lower than that of liquid inoculum. Up to now the project has used liquid material which is harvested once the algal density has reached the required density. Culture is not synchronized, so every 1 or 2 days there is a pond ready for material to be collected, transported and inoculated.

From 2011 onwards, a Chinese private enterprise company will support the Wuhan team to extend this technique to another 133 km^2 in the next 5 years. The cost has to be reduced much more, as the present cost is ca. 0.15¥ per m^2, without taking into consideration the building expenses for the culture ponds. The costs include the salary of occasional workers, electricity, water and transport up to a distance of 300 km.

In spite of the concern about cost, the technique has many advantages, with the formation of a biological soil crust being speeded up markedly following inoculation. Changes measured by Wang et al. (2009) were increases in soil organic carbon, total N; total salt, calcium carbonate, conductivity, crustal thickness, cohesion and chlorophyll a content. Other reported changes include increases in soil enzyme activities with inoculation time (Tang et al. 2007b), an enhanced net CO_2 exchange rate (Maestre et al. 2006), appearance of a few moss species in the second year (Wang et al. 2009) and establishment of grasses and shrubs (Wang et al. 2009). All this has convinced researchers that the technology is effective in the recovery of biological soil crusts and the restoration of soil ecosystems (Maestre et al. 2006; Wang et al. 2009). Liu et al. (2008) have even suggested that the use of inocula could be tried in controlling dust on the moon and Mars and this idea was considered in more detail by Cockell (2010). Belnap (2003) speculated on the importance of cyanobacterial crusts in the development of soils in the early history of the earth, while Beraldi and Farmer (2010) provided evidence which seems to support this. Biogenic features of the Dripping Stone Quatrzite, a siliciclastic formation in Arizona compared closely to modern biological soil crusts.

12.4.2 Soil Fertilization

Because cyanobacteria have an important role in many soil ecosystems, it is also possible to improve soil characteristics by modifying cyanobacterial populations in less extreme

situations than the deserts discussed above. Many of the ways in which cyanobacteria can influence soil were considered by Whitton (2000), but increasingly the information about these processes at particular sites is becoming more quantitative. Among more recent studies are ones on increases in available combined N resulting from cyanobacterial nitrogenase (Aranibar et al. 2003; Tirkey and Adhikary 2005), increases in available P, K, Mg, Ca, Mn and Zn (Pardo et al. 2009), total organic carbon, soil organic carbon, cation-exchange capacity, water infiltration (Warren 2001), water-holding capacity, hydraulic conductivity, mean weight diameter (Nisha et al. 2007; Obana et al. 2007), porosity (Issa et al. 1999), improved soil aggregation and seedling emergence (Rogers and Burns 1994). All of these have the potential for helping to reclaim degraded soil ecosystems (Obana et al. 2007). More specifically, cyanobacterial activity can cause agglomeration of soil particles and improve the tilth, while their application as biofertilizer and soil conditioner can reduce the use of chemical fertilizers for sustainable agriculture (Malliga and Subramanian 2002; Ibraheem 2007).

Caution must be used in trying to extrapolate results from one region to another, but the detailed information in some accounts can provide much of interest for projects elsewhere. Issa et al. (2007) reported a laboratory study on the effect of a *Nostoc* inoculum on a 1-cm layer of poorly aggregated soil from the Eastern Cape, South Africa. The *Nostoc*, which was grown from a strain isolated in Tanzania producing a large amount of EPS, was sprayed on the surface of one set of samples at an inoculum density of 3 $g\,L^{-1}$, leading to a dense superficial network of *Nostoc* and its EPS after 4–6 weeks. There was a rapid improvement in aggregate stability and this increased gradually with time and cyanobacterial growth. While it is clear that the cyanobacterium was responsible for the increased aggregation, no experiments were included to see if an equivalent addition of nutrients would have eventually stimulated growth of local strains of N_2-fixing cyanobacteria. Acea et al. (2003) tested the effect of cyanobacterial inocula on heated soil samples from N-W. Spain, which represented a sort of temporary desert that might occur after a fire. Four strains were used (*Oscillatoria*, *Nostoc*, *Scytonema* or a mixture). Inoculation induced great microbial proliferation near the surface of the heated soils, together with high increases in organic matter and nutrients. In general, cellulolytics were increased by four logarithmic units, amylolytics and ammonifiers by three logarithmic units and nitrifiers by more than two logarithmic units. No explanation was suggested for the marked increase in cellulolytics, when the cyanobacteria themselves would not have formed cellulose. Among the available nutrients the highest increase was for Ca, followed, respectively, by Mg, K, Na and P.

The authors concluded that inoculation of burned soils with N_2-fixing cyanobacteria was a very useful biotechnological approach to promoting microbiotic crust formation, enhancing C and N cycling microorganisms and increasing organic matter and nutrient contents in soils damaged by heating. It is unclear whether the most suitable strains were selected for the soils in this study, so there is considerable potential for developing the method in regions prone to fire risk. It should be easy to store suitable inocula on a sufficiently large scale to deal with problems when they occur.

The microbial cover can transform the region from an accumulation zone of water to a source zone with consequences on regional fertility transfer (Valentin et al. 2004). Reclamation is not just an accumulation process, because excess ammonium and nitrate can be leached away (Veluci et al. 2006). Addition of other material can sometimes speed the rate of development of the cyanobacterial community, such as the incorporation of pyrite, as tested by Pandey et al. (2005).

Although the extensive literature on the use of cyanobacteria and *Azolla* in rice-fields to enhance nitrogen fixation and reduce or even avoid the need for nitrogen fertilizer lies outside the scope of this chapter, it does suggest guidelines for research on the use of inocula in the development of biological crusts in arid regions. Some, though not all, the earlier research on biofertilizer in rice-fields was rather uncritical and sometimes led to conflicting results (Whitton 2000). While studies at some sites showed that soil inoculation had no effect on rice yield (e.g. Reddy et al. 1986), others did seem to provide convincing evidence of positive effects of adding cyanobacteria on the growth, yield and mineral composition of rice plants (Dhar et al. 2007; Tripathi et al. 2008). Soil inoculation also reduced the absorption by rice of Cd, Ni and As (Tripathi et al. 2008). One of the most important findings is convincing evidence for the need to use local cyanobacterial strains (Nisha et al. 2007; Swarnalakshmi et al. 2007), something that was all too often ignored in early studies on rice fields.

In arid and semiarid areas the uptake of water from the atmosphere serves as an important water source for biological soil crusts, and also vascular plants, insects and small animals. For instance, in the Qubqi Desert water uptake from the atmosphere accounted for 25.0–39.8% of total water uptake was obtained by a water vapour adsorption mechanism, the remainder being from the soil (Lan et al. 2010a). The formation of crusts promoted water uptake, but the increased uptake did not occur immediately after inoculation or crust formation. The water taken from the atmosphere increased significantly from day 15 after inoculation and the soil water content was markedly enhanced from day 20. The authors concluded that the growth of algal filaments and their secretions were the main factors increasing the rate of water uptake by the crusts and the resulting higher water content.

12.4.3 Food Exploitation

Although "Spirulina" (*Arthrospira*) is the cyanobacterium which has acquired the widest acceptance as a high nutrient food (Chaps. 25 and 26), three species of *Nostoc, N. flagelliforme*, *N. commune* and *N. sphaeroides*, have long been considered another good food; these have been a traditional delicacy in China and other parts of east and south-east Asia for many centuries. *N. flagelliforme* is the one most closely associated with semi-desert regions and its biology and practical use were reviewed by Gao (1998). The method of harvesting material with rakes was causing such environmental damage in Inner Mongolia and adjacent provinces of China, that harvesting was banned, with marked sociological effects (Roney et al. 2009). This in turn stimulated research on its possible commercial large-scale culture. Its developmental stages in indoors have been described in some detail (Gao and Ye 2003; Liu and Chen 2003; Su et al. 2007), but so far no breakthrough has been achieved in culture techniques permitting large-scale culture. Further research is needed to establish how to get rapid growth while providing what may prove to be an obligate requirement, the alternating wet and dry conditions mentioned earlier. It may also be possible eventually to combine methods for the development of biological soil crusts with ones that permit growth of *N. flagelliforme* without harvesting leading to the crusts being damaged.

The macroscopic colonies of *Nostoc commune* are found world-wide, although the name is known to include genetically diverse organism (Potts 2000). It is easy to collect large amounts from many sites world-wide, where it is subject to alternate wetting and drying, though typically a greater proportion of the time in the wet state than *N. flagelliforme*, which suggests that it should be easier to develop large-scale culture methods for *N. commune* if it proved economically worthwhile to do so. Nevertheless Chen et al. (2011) established laboratory conditions where showed a 250% increase in *N. flagelliforme* in 25 days under laboratory conditions with much lower temperatures and light flux than likely to occur in natural populations in summer, so it may prove economically possible to grow this species as well. *N. sphaericum* is the species most associated with wet environments and its life cycle was described by Becerra-Absalon and Tavera (2009).

Mass culture of the near-spherical colonies has been achieved at the Institute of Hydrobiology, Wuhan, and the product is being widely sold in markets in China, with a good profit. Nevertheless, it is important to remember the possibility of toxic strains when developing methods for large-scale culture of *Nostoc*. All 21 *N. commu*ne samples bought at markets in Peru showed the presence of β-N-methylamino-L-alanine (BMAA), a neurotoxic amino-acid (Johnson et al. 2008) The diversity of forms, especially those named *N. commune*, means that every species or strain grown for food should be tested rigorously before it is marketed. *Nostoc* culture is considered further in Chap. 26.

12.4.4 Utilization of Exopolysaccharides

The importance of cyanobacterial EPS in biological crust formation and improving soil structure has already been mentioned several times and the biological roles for the organisms are discussed in Chap. 18. Pereira et al. (2009) reviewed its composition, function and factors affecting synthesis and an investigation of the factors important in its formation by *Microcoleus vaginatus* was reported by Xie et al. (2008). Many studies have reported on how the various properties could be put to practical use. There is information about protection from desiccation, tolerance to salt and alkali, antibiotics, ultraviolet radiation (Mazor et al. 1996; Stal 2000; Chen et al. 2002, 2003a, b; Tang et al. 2007a), increase in crust cohension (Hu et al. 2002b), the formation of soil aggregates, utilization of phosphorus and release of trace elements, and removal of heavy metals (De Philippis et al. 2001, 2003, 2007; Micheletti et al. 2008) and immobilization of enzymes, especially phosphomonoesterase (Whitton et al. 2005). These indicate the possible uses of EPS in amelioration of desert soil (Painter 1993) and removal of heavy metals from polluted water (De Philippis et al. 2003, 2007; Micheletti et al. 2008), whether as isolated EPS or by growth of organisms with a high EPS content.

The most important feature of EPS is its high and varied bioactivity (Garbacki et al. 2000). In order to understand and perhaps exploit the EPS of edible *Nostoc* their chemical properties have been analyzed in some detail (Huang et al. 1998; Helm et al. 2000). Several desert strains have been used for the analysis of monosaccharide linkages, chemical structure and physical properties (Hu et al. 2003a; Hokputsa et al. 2003). Other studies include ones on anti-tumour and anti-inflammatory activities (Garbacki et al. 2000; Zhang et al. 2008) and the positive nematicidal potential of culture filtrates of *Microcoleus vaginatus* (Khan et al. 2005).

12.4.5 Other Substances of Potential Use

Because cyanobacteria in arid environments are under stress, it seems likely that these will prove an important source of molecules with potentially useful properties. Among the many studies investigating the effects of cyanobacterial extracellular materials on other organisms, soil isolates were tested by Safonova and Reisser (2005) and terrestrial ones by Svircev et al. (2008). A number of researchers have shown an interest in possible uses for the photoprotectants, scytonemin and the mycosporine-like amino acids (e.g. Kulik 1995; Rastogi and Sinha 2009). Scytonemin production

increases in cyanobacteria which can form the molecule (Harel et al. 2004; Fleming and Castenholz 2008; Pattanaik et al. 2002: see Chap. 19). Genomic and gene analysis of scytonemin biosynthesis in *N. punctiforme* has been carried out (Soule et al. 2007, 2009) and the initial biosynthetic steps established (Balskus and Walsh 2008).

12.5 Practical Methods

12.5.1 Stabilization of Topsoil

12.5.1.1 Wind-Tunnel Testing

The effects of wind speed have been tested on both individual colonies and soil crusts. A wind of 2.0 m s^{-1} led to the mass of *Nostoc flagelliforme* colonies decreasing to 50% 2.8 times faster than in still air, while a further rise to 3.4 m s^{-1} led to it being 4.9 times faster; this was the stage at which photosynthetic efficiency started to be affected (Gao et al. 1998). Proper wind tunnels are needed for tests on the stability of soils against wind erosion and experiments have been conducted on both biological and physical crusts (Liu et al. 1994; McKenna-Neuman et al. 1996; Hu et al. 2002a). Earlier studies (Liu et al. 1994; McKenna-Neuman et al. 1996) expressed results as the change in mass of an eroded soil, so the data failed to reflect actual cohension of the topsoil. The method has now been modified to express the results on an areal basal – the portion of erosion area affected (Hu et al. 2002a). Another problem is that natural crusts are often a mixture of cyanobacteria, eukaryotic algae, fungi, lichens and mosses in varying proportions, so it is necessary to make a cyanobacterial crust in order to measure cyanobacterial cohesion. Test are carried out in a straight-line suction wind tunnel, which provides laminar air flow at a slow velocity.

The practical methods are as follows.

Preparation of artificial algal crust

1. Fill flat rectangular containers (e.g. 30×40×2.8 cm trays with small holes at the bottom for drainage) with unconsolidated local sand to a depth *ca*. 5 mm below the height of containers. The surface of the sand is levelled by drawing a straight bar along the edges of the top of the tray from end to end, then soaked with water, but without any water accumulating on the surface.
2. Inoculation. A slurry of ground and homogenized cyanobacterial material is sprayed onto the sand surface for a fixed time using a water jug with spray nozzle. Four replicates are used for each treatment in every experiment. The containers are then moved outdoors or to a greenhouse for at least a week in order to be 'trained' – an essential step. If a greenhouse is used, it should have natural light conditions (canopy of transparent plastic sleeting). The final water content and cyanobacterial biomass of air-dried sands are determined before the wind tunnel experiments by subsampling the plots outside the test area (Hu et al. 2002a). The control surfaces are not inoculated, but are treated similarly with water or BG11 medium.

Wind-tunnel test

1. Sufficient local unconsolidated sand is delivered into the air stream at the entrance of the tunnel working section, upstream of the crust surface.
2. The floors of the working section of the tunnel is covered with a thin wood sheet and the containers are placed in the tunnels with their long dimensions parallel to the tunnel. The cyanobacterial crusts are level with the tunnel ground to maintain laminar, non-turbulent flow.
3. The degree of wind erosion is expressed as % of a 30×30 cm square showing damage to the crust. Wind speed is measured with a pitot tube, with different speeds tested during the course of the studies. Prior to testing the crusts, the effects of very low moisture on threshold friction velocity are tested, so that the effect of moisture can be separated from the effect of cyanobacterial growth.

Field data can be used to indicate the wind velocity required for the initiation of saltation, the threshold friction velocity (TFV), the aerodynamic roughness length and the wind profile above sand surfaces covered by biological crusts. The drawback of this laboratory tunnel is that it cannot be moved easily. Basis on a similar principle, a portable wind tunnel was invented, which has been used by Belnap and Gillette (1998) and Eldridge and Leys (2003) for many studies. They found TFVs of undisturbed crusts well above the wind forces experienced at the sites and the cover of biological crusts was significantly positively correlated with dry aggregation levels greater than 0.85 mm.

12.5.1.2 Press-Resistance Testing

As well-developed natural crusts can withstand more than a 25 m s^{-1} sand-holding wind, the method of sand wind tunnel is unable to reflect differences in their strength (Hu et al. 2002a), so testing the press-tolerance strength is very important. The press-resistance cohesion can measure by using a penetrometer (A-0152), which has been used in a number of studies (Hu et al. 2002a). The calculated formula is

$$\rho = 100X / 0.7952\left(40 - X\right)^2$$

where ρ is press-resistance cohesion (g cm^{-2}), X is recoiled length (mm).

Concerning this instrument, an important question is the angle of penetration.

12.5.1.3 Flexure Testing

The flexure test was invented and used by McKenna-Neuman et al. (1996), the principle of apparatus being to test the blending moment resisted by interial stresses set up in the crust. The modulus of elasticity for a load is determined from

$$E = Wl^3/48I(\Delta y)$$

where W is load at the elastic limit, l is length of the crust, I is the moment of intertia, Δy is the deflection of the crust in the direction of the applied force.

As samples need to be collected and separated from the soil below when measured, the results of such flexure tests may not always be suitable for assessing cohesion *in situ*. Although direct comparisons have not yet been made, we suggest that flexure testing provides more realistic information for biological soil crusts, while the penetrometer is more useful for physical crusts. Langston and Neuman (2005) found that strong salt crusts tested with a penetrometer were easier to break down and erode than relatively weak biotic crusts. This may be the reason why the relationship between algal biomass and press-cohesion is not as tight as that of the cohesion against wind erosion (McKenna-Neuman et al. 1996; Belnap et al. 2007; Xie et al. 2007). Inorganic minerals and soil texture are mainly affected by press cohesion, organic matter and biological components by flexure cohesion, whereas the data from wind tunnel studies reflect an integrated cohesion (Hu et al. 2002b).

12.5.1.4 Visual Technique

In order to assess conveniently the stability of cyanobacterial dominated crusts, Belnap et al. (2008) put forward a visual technique based on soil surface darkness. The research team at Wuhan also had realized this phenomenon and used the principle when collecting samples (Hu et al. 2003c), and found there was high repetition in the results. Two problems remain to be solved. (1) The effect of soil texture and light regime on darkness of the crust needs to be validated further. (2) How the index categories can be applied under different climatic still needs to be investigated.

12.5.2 Assessment of Biomass

Selecting the best ways to measure biomass has been a persistent and complex problem throughout the study of terrestrial algae. Some of the earlier problems and successes were included in the review by Whitton (2000). Hu and Liu (2003b), who attempted to compare direct counts, dilution plate techniques and measurements of biovolume and chlorophyll a, then put forward a relatively exact method to quantify algal biomass to the species level. This was a version of the biovolume technique, but it is time-consuming and rather inconvenient. When studying saline land in Japan, Tsujimura et al. (2000) concluded that culture dilution was suitable for spatial, but not seasonal studies. Prasanna et al. (2006) developed the most probable number method to isolate and count cyanobacteria simultaneously. This was a more elaborate approach to a procedure which has sometimes been used in rice-field studies. It involved the use of 96-well plates holding 0.3 mL per well. The technique could also be used for isolating cyanobacteria by isolating colonies in the gel matrix; sampled could be stored at room temperature without loss of viability for 5–6 weeks. This technique was suitable for large sample sizes. Nevertheless the Wuhan team prefers direct observation or a combination of direct counting and molecular analysis; enrichment culture is used just for identification.

Chlorophyll a content is commonly used as an indicator of cyanobacterial biomass (Bowker et al. 2002), while the presence of chlorophyll b in addition can provide an estimate of crust recovery, with an increasing ratio between the two indicating an increasing contribution by green algae and mosses (Rychert 2002). Pigment extraction is a particular problem with crusts, because of the range of other pigments that may be present, especially those associated with lichens, becomes an increasing problem as crusts develop. Lan et al. (2011a) reported significant differences in the efficiency of various organic solvent. Dimethyl sulphoxide (DMSO) provided the highest extraction efficiencies, but was unsuitable for crusts containing a substantial proportion of other pigments. N,N-dimethyl-formamide not only showed low extraction efficiencies, but also the efficiency was easily affected by interfering pigments in well-developed crusts. In general, ethanol was more effective than acetone and also provided more stability and greater safety. Methanol has been used in many previous studies on chlorophyll extraction of dried cyanobacterial samples, usually following the procedures of Marker et al. (1980) and Marker (1995). However, its use for the projects at Wuhan and elsewhere in China has largely ceased because of its greater toxicity than ethanol.

12.6 Desert Endoliths

When conditions become too severe for cyanobacteria to grow in soils, they are restricted to chasmolithic, endolithic or hypolithic growths (see Chap. 10) associated with rocks. In extreme cases, such as in Beacon Valley in the McMurdo Dry Valleys, Antarctica, studied by Pointing et al. (2007, 2009; Sect. 14.2.1), rocks provide isolated "islands" of colonization, as soils in the more extreme environments appear to be free of phototrophs. Deserts with translucent rocks almost always have endolithic or hypolithic cyanobacterial growths, though the colonization frequency in the most arid regions may be very low (see below). In the case of deserts where quartz forms a pavement, the cyanobacterial layer occurs under the pavement i.e. truly hypolithic. Such communities have been described from the Mojave Desert (Schlesinger et al. 2003) and the deserts in China studied by Warren-Rhodes et al. (2007a, b) and also several regions in Antarctica (Broady 2005; Wood et al. 2008). The growths are usually dominated by the form-genus *Chroococcidiopsis*, which is extremely resistant to ionizing radiation and desiccation (Billi et al. 2000; Cockell et al. 2005). Desert sandstones, which typically have chasmoliths as well as endoliths, usually also have *Chroococcidiopsis* morphotyes. Phylogenetic

analyses of *Chroococcidiopsis* populations from deserts in 18 different regions of the world showed no evidence of recent inter-regional gene flow, indicating that populations have not shared common ancestry since before the formation of modern continents (Bahl et al. 2011). The authors concluded that the global distribution of desert cyanobacteria has not resulted from widespread contemporary dispersal, but is an ancient evolutionary legacy. However, their study dealt only with Chroococcidiopsis, perhaps the organism most closely adapted to the desert environment, so caution should be used in generalizing to other genera until phylogenetic studies have been done on them.

In contrast to most studies of desert quartz, Pointing et al. (2007) found that quartz in the McMurdo Dry Valleys of the Antarctic is dominated by Oscillatoriales; in this case the range of cyanobacterial genera increased as conditions become slightly more favourable. The communities are usually visible as green layers or clumps, but Lacap et al. (2011) reported that some hypolithic communities in the Atacama Desert are red due to dominance by diverse chloroflexi, which they suggested might occur under more nutrient-rich conditions.

The extent to which individual translucent rocks have cyanobacterial communities differs markedly between sites. Near-100% colonization was reported for the warm Mojave Desert (Schlesinger et al. 2003). A small-scale ecological survey in the central southern region of the Tibet Autonomous Region in China (29°07′ N, 85°05′E, alt. 4,638 m) found that 36% of quartz rocks were colonized. Lower values occur in the Taklimakan and Qaidam Basin deserts of western China, where colonization of the available habitat ranged from 0.37% to 12.6%, with the lowest values at cold dry desert sites (Warren-Rhodes et al. 2007b). In the last study differences between sites were most strongly correlated with moisture-related variables, but patchiness within sites was correlated with local geology, there being more frequent colonization on large rocks; dispersal during rainfall was also an important factor. In the study of the hyper-arid core of the Atacama Desert by Schlesinger et al. (2003), the frequency of colonization was very low – only 3 out of 3,723 stones in an area >2,000 m^2. Further studies on the limits for cyanobacterial growth in the Atacama Desert have been reported by Warren-Rhodes et al. (2006) and Wierzchos et al. (2006). *Chroococcidiopsis* morphotypes were absent at the most extreme arid sites, but one was recorded in halite evaporite rocks in the driest part of the desert, where not even quartz rocks were colonized (Wierzchos et al. 2006). Warren-Rhodes et al. (2006) concluded that hypoliths in the Atacama are very long-lived, possibly persisting for thousands of years. However, disturbed hypoliths seldom survive, indicating the importance of the substrate being stable (Lacap et al. 2011).

Several studies have provided detailed accounts of diversity at particular sites using molecular techniques. In the case of the quartz pavement in central Tibet, where a cold desert tundra environment has persisted over a geological timescale, profiling using terminal restriction fragment length polymorphism revealed no significant difference in community structure between rocks (Wong et al. 2010). The hypolithon was dominated by cyanobacterial phylotypes, with relatively low frequencies of other bacterial phylotypes. In addition to four *Chroococcidiopsis* clones, the cyanobacteria included many filamentous ones (*Nostoc*, *Leptolyngbya*, *Phormidium*, *Pseudanabaena*, *Oscillatoria*, *Microcoleus*, *Plectonema*).

Among the many questions still requiring clear-cut answers is the extent to which maintenance of populations in endolithic communities depends on long-lived cells repairing stress damage or whether there is a turn-over of cells, with those dying being replaced by new cell growth. This was investigated by Billi (2009) for a laboratory strain of *Chroococcidiopsis* (CCMEE 029), which had been stored in a desiccated state for 4 years. Live and dead cells were found in dried cell aggregates and the author suggested that subtle modifications to the cell environment are required to dry without dying. The cells avoiding or reducing subcellular damage showed a variety of responses, including avoiding or limiting genome fragmentation and genome covalent modifications, and preserving intact cell surface membranes and phycobiliprotein autofluorescence.

The importance of nutrient cycling within the sandstones of the Beacon Valley was made clear by Banerjee et al. (2000b), who compared phosphomonoesterase and phosphodiesterase activities of the endolithic communities from three different types of rock, which had three different dominants: *Chroococcidiopsis*, mixed *Gloeocapsa* and the green alga *Trebouxia*. Although some dead cells were observed (B.A.W., unpublished), no attempt was made to compare the ratio of live to dead for the three genera. The optimum pH values for phosphomonoesterase activity were 9.5, 5.5 and 8.0, respectively, all of which corresponded quite closely to the pH values of the three aqueous rock extracts. The communities showed significantly higher phosphomonoesterase activity at 5°C than 1°C, and two of them showed much higher activity at 5°C than 10°C. All three had slightly lower activity in the light (8 µmol photon m^{-2} s^{-1}) than the dark and prior exposure to light also led to lower subsequent activity in the dark. However, an axenic isolate of *Chroococcidiopsis* showed various physiological differences from that of the same organism in crushed rock fragments, especially a much higher temperature optimum (Banerjee et al. 2000a); this suggests that the physiology of the organized is influenced by being immobilized within the rock. As P is scarce in the Beacon Sandstone and more likely to be a limiting element for growth than C or N, its cycling within a particular rock type is probably a key factor influencing metabolic activity of the community.

Another unanswered question is the extent to which *Chroococcidiopsis* fixes nitrogen in these environments.

Friedmann and Kibler (1980) concluded that polar and other endolithic cyanobacteria largely used abiotic nitrogen sources, but N_2 fixation was reported in *Chroococcidiopsis*-dominated communities endolithic in gypsum in an alpine environment (Boison et al. 2004) and all eight strains of *Chroococcidiopsis* mentioned by Rippka et al. (2001) synthesized nitrogenase under anoxic conditions. In view of the phylogenetic diversity of the form-genus *Chroococcidiopsis* (Billi et al. 2010; Bahl et al. 2011), it will require studies on a wide range of communities before the situation is clear.

12.7 Conclusions

There is now a great deal of information about cyanobacteria and other microalgae in desert and semi-desert regions, especially in areas where at least part of the surface is covered in biological soil crusts. The role of these crusts in stabilizing soils, reducing desertification and in some cases helping to reverse it, is well established. Examples of reclamation are increasing, especially in China, and the review makes clear the importance of research on the biology of the crusts and the practical methods for encouraging their development, including inoculation and minimizing damage by trampling and livestock. Most accounts mention the importance of cyanobacteria, especially during the early stages. However, it is still difficult to generalize on the organisms and processes leading to biological soil formation under different types of desert environment – for instance those dependent on rare rainfall events versus those dependent on dew formation, or the effects of different light regimes. It is essential to be able to do this, if the research and practical experience gained in countries such as China are to be applied to parts of the world like East Africa, where steps to reduce desertification are urgently needed, yet there is little local scientific knowledge or skill to put them into practice. Greater standardization of sampling and analytical methods, together with a more critical statistical evaluation of the data would make it easier to generalize between studies.

References

Acea MJ, Prieto-Fernández A, Diz-Cid N (2003) Cyanobacterial inoculation of heated soils: effect on microorganisms of C and N cycles and on chemical composition in soil surface. Soil Biol Biochem 35:513–524

Agrawal SC, Pal U (2003) Viability of dried vegetative cells or filaments, survivability and/or reproduction under water and light stress, and following heat and UV exposure in some blue-green and green algae. Folia Microbiol 48:501–509

Agrawal SC, Singh V (2000) Vegetative survival, akinete formation and germination in three blue-green algae and one green alga in relation to light intensity, temperature, heat shock and UV exposure. Folia Microbiol 45:439–446

Aranibar JN, Anderson IC, Ringrose S, Macko SA (2003) Importance of nitrogen fixation in soil crusts of southern African arid ecosystems: acetylene reduction and stable isotope studies. J Arid Environ 54(2):345–358

Arnold RJ, Convey P, Hughes KA, Wynn-Williams DD (2003) Seasonal periodicity of physical factors, inorganic nutrients and microalgae in Antarctic fellfields. Polar Biol 26(6):396–403

Bahl J, Lau MCY, Smith DLD, Vijaykrishna D, Cary SC, Kumberley D, Warren-Rhodes AW, Wong FKY, McKay CP, Pointing SB (2011) Ancient origins determine global biogeography of hot and cold desert cyanobacteria. Nat Commun 2:163. doi:10.1038/ncomms1167

Balskus EP, Walsh CT (2008) Investigating the initial steps in the biosynthesis of cyanobacterial sunscreen scytonemin. J Am Chem Soc 130:15260–15261

Bamforth SS (2008) Protozoa of biological soil crusts of a cool desert in Utah. J Arid Environ 72(5):722–729

Banerjee M, Whitton BA, Wynn-Williams DD (2000a) Surface phosphomonoesterase activity of a natural immobilized system: *Chroococcidiopsis* in an Antarctic desert rock. J Appl Phycol 12:549–552

Banerjee M, Whitton BA, Wynn-Williams DD (2000b) Phosphatase activities of endolithic communities in rocks of the Antarctic Dry Valleys. Microb Ecol 39:80–91

Becerra-Absalon I, Tavera R (2009) Life cycle of *Nostoc sphaericum* (Nostocales, Cyanoprokaryota) in tropical wetlands. Nova Hedwig 88(1–2):117–128

Belnap J (1993) Recovery rates of cryptobiotic crusts: inoculant use and assessment methods. Gt Basin Nat 53:89–95

Belnap J (2003) The world at your feet: desert biological soil crusts. Front Ecol Environ 1:181–189

Belnap J, Gardner JS (1993) Soil microstructure in soils of the Colorado Plateau: the role of the cyanobacterium *Microcoleus vaginatus*. Gt Basin Nat 53:40–47

Belnap J, Gillette.DA (1998) Vulnerability of desert biological soil crusts to wind erosion: the influences of crust development, soil texture, and disturbance. J Arid Environ 39:133–142

Belnap J, Lange OL (eds) (2001) Biological soil crusts: structure, function, management. Springer, Berlin, 503 pp

Belnap J, Büdel B, Lange OL (2001) Biological soil crusts: characteristics and distribution. In: Belnap J, Lange OL (eds) Biological soil crusts: structure, function, management. Springer, Berlin, p 1et seq, 503 pp

Belnap J, Phillips SL, Herrick JE, Johansen JR (2007) Wind erodibility of soils at Fort Irwin, California (Mojave Desert), USA, before and after trampling disturbance: implications for land management. Earth Surf Process Landf 32:75–84

Belnap J, Phillips SL, Witwicki DL, Miller ME (2008) Visually assessing the level of development and soil surface stability of cyanobacterially dominated biological soil crusts. J Arid Environ 72: 1257–1264

Beraldi H, Farmer JD (2010) Evidence of subaerial environments in the Mesoproterozoic Dripping Spring Quartzite, Apache Group, Arizona. Geol Soc Am Abstr Program 42(5):430

Berard A, Dorigo U, Humbert JF, Martin-Laurent F (2005) Microalgae community structure analysis based on 18S rDNA amplification from DNA extracted directly from soil as a potential soil bioindicator. Agron Sustain Dev 25:285–291

Bhatnagar A, Bhatnagar M (2005) Microbial diversity in desert ecosystems. Curr Sci 89:91–100

Bhatnagar A, Makandar MB, Garg MK, Bhatnagar M (2008) Community structure and diversity of cyanobacteria and green algae in the soils of Thar Desert (India). J Arid Environ 72:73–83

Billi D (2009) Subcellular integrities in *Chroococcidiopsis* sp. CCMEE 029 survivors after prolonged desiccation revealed by molecular probes and genome stability assays. Extremophiles 13(1):49–57

Billi D, Friedmann EI, Hofer K, Grilli-Caiola M, Ocampo-Friedmann R (2000) Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. Appl Environ Microbiol 66:1489–1492

Billi D, Wilmotte A, McKay CP (2010) Desert strains of *Chroococcidiopsis*: a platform to investigate genetic diversity in extreme environments and explore survival potential beyond Earth. European Planetary Science Congress 5: EPSC2010-267, Rome, 2 pp

Boison G, Mergel A, Jolkver H, Bothe H (2004) Bacterial life and dinitrogen fixation at a gypsum rock. Appl Environ Microbiol 70:7070–7077

Booth WE (1941) Regeneration of abandoned fields in Kansas and Oklahoma. Am J Bot 28:415–422

Bowker MA (2007) Biological soil crust rehabilitation in theory and practice: an underexploited opportunity. Restor Ecol 15:13–23

Bowker MA, Reed SC, Belnap J, Phillips SL (2002) Temporal variation in community composition, pigmentation, and Fv/Fm of desert of cyanobacterial soil crusts. Microb Ecol 43:13–25

Boye-Petersen J (1923) The freshwater Cyanophyceae of Iceland. In: Rosenvinge LK, Arming EW (eds) Arb Bot Kobenhavn (The Botany of Iceland 2) 101(7):251–324

Boyer SL, Johansen JR, Flechtner VR, Howard GL (2002) Phylogeny and genetic variance in terrestrial *Microcoleus* (Cyanophyceae) species based on sequence analysis of the 16s rRNA gene and associated 16S-23S ITS region. J Phycol 38:1222–1235

Branco LHZ, Hoffmann L, Teixeira JP, Ferreira V, de Morais JC (2009) Aerophytic cyanoprokaryotes from the Atlantic rainforest region of Sao Paulo State, Brazil: Chroococcales and Oscillatoriales. Cryptogam Algol 30(2):135–152

Broady PA (2005) The distribution of terrestrial and hydro-terrestrial algal associations at three contrasting locations in southern Victoria Land, Antarctica. Algol Stud 118:95–112

Büdel B (2005) Microorganisms of biological crusts on soil surfaces. Soil Biol 3(V):307–323

Büdel B, Veste M (2008) Arid zone ecosystems. Ecol Stud 208B:149–155

Büdel B, Darienko T, Deutschewitz K, Dojani S, Friedl T, Mohr KI, Salisch M, Reisser W, Weber B (2009) Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. Microb Ecol 57:229–247

Chen LZ, Liu YD, Song LR (2002) The function of exopolysaccharides of *Microcoleus* in the formation of desert soil. Acta Hydrobiol Sin 26(2):155–159

Chen LZ, Li DH, Liu YD (2003a) Salt tolerance of *Microcoleus vaginatus* Gom., a cyanobacterium isolated from desert algal crust, was enhanced by exogenous carbohydrates. J Arid Environ 55:645–656

Chen LZ, Liu YD, Li DH (2003b) Effect of salt stress on physiological and biochemical characteristics of *Scytonema javanicum*. J Desert Res 23:285–288

Chen LZ, Xie ZM, Hu CX, Li YD, Wang GH, Liu YD (2006a) Man-made desert algal crusts as affected by environmental factors in Inner Mongolia. China J Arid Environ 67:521–527

Chen LZ, Li DH, Song LR, Hu CX, Wang GH, Liu YD (2006b) Effects of salt stress on carbohydrate metabolism in desert soil alga *Microcoleus vaginatus*. J Integr Plant Biol 48(8):1–5

Chen LZ, Xie ZM, Li DH, Liu YD, Wang GH (2006c) Recovery of photosynthetic activity of *Microcoleus vaginatus* after water loss and rehydration. Acta Hydrobiol Sin 30(4):404–407

Chen LZ, Wang GH, Hong S, Liu A, Li C, Lui Y-D (2009a) UV-B induced oxidative damage and protective role of exopolysaccharides in desert cyanobacterium *Microcoleus vaginatus*. J Integr Plant Biol 51(2):194–200

Chen XF, Jia SR, Yue SJ, Wang N, Li CT, Wang Y (2009b) Effect of solid bed-materials on growth of vegetative cells of *Nostoc flagelliforme*. J Appl Phycol 22:341–347

Chen XF, Jia SR, Yue SJ, Wang Y, Wang N (2011) Biological crust of *Nostoc flagelliforme* (cyanobacteria) on sand bed materials. J Appl Phycol 23:67–71

Cockell CS (2010) Geomicrobiology beyond Earth: microbe-mineral interactions in space exploration and settlement. Trends Microbiol 18(7):308–314

Cockell CS, Schuerger AC, Billi D, Friedmann EI, Panitz C (2005) Effects of a simulated Martian UV flux on the cyanobacterium, *Chroococcidiopsis* sp. 029. Astrobiology 5:127–140

Cox PA, Richer R, Metcalf JS, Banack SA, Codd GA, Bradley WG (2009) Cyanobacteria and BMAA Exposure from desert dust: a possible link to sporadic ALS among Gulf War veterans. Amyotroph Lateral Scler 10(Suppl 2):109–117

Dana ED, Mota JF (2006) Vegetation and soil recovery on gypsum outcrops in semi-arid Spain. J Arid Environ 65(3):444–459

Darby BJ, Housman DC, Zaki AM, Shamout Y, Adl S, Belnap J, Neher DA (2006) Effects of altered temperature and precipitation on desert protozoa associated with biological soil crusts. J Euk Microb 53:207–514

Darby BJ, Neher DA, Belnap J (2007) Soil nematode communities are ecologically more mature beneath late- than early-successional stage biological soil crusts. Appl Soil Ecol 35:203–212

Davey MC, Clarke KJ (1992) Fine structure of a terrestrial cyanobacterial mat from Antarctica. J Phycol 28:199–202

De Philippis R, Sili C, Paperi R, Vincenzini M (2001) Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. J Appl Phycol 13:293–299

De Philippis R, Paperi R, Sili C, Vincenzini M (2003) Assessment of the metal removal capability of two capsulated cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936. J Appl Phycol 15: 155–161

De Philippis R, Paperi R, Sili C (2007) Heavy metal sorption by released polysaccharides and whole cultures of two exopolysaccharide-producing cyanobacteria. Biodegradation 18:181–187

Dhar DW, Prasanna R, Singh BV (2007) Comparative performance of three carrier based blue green algal biofertilizers for sustainable rice cultivation. J Sustain Agric 30(2):41–50

Drouet F (1981) Summary of the classification of blue-green algae. Beih Nova Hedwig 66:133–209

Eldridge DJ, Leys JF (2003) Exploring some relationships between biological soil crusts, soil aggregation and wind erosion. J Arid Environ 53:457–466

Flechtner VR (2007) North American microbiotic soil crust communities: diversity despite challenge. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Berlin, pp 537–551, 811 pp

Flechtner VR, Boyer SL, Johansen JR, DeNoble ML (2002) *Spirirestis rafaelensis* gen. et sp. nov (Cyanophyceae), a new cyanobacterial genus from arid soils. Nova Hedwig 74(1–2):1–24

Flechtner VR, Johansen JR, Belnap J (2008) The biological soil crusts of the San Nicolas Island: enigmatic algae from a geographically isolated ecosystem. West N Am Nat 68(4):405–436

Fleming ED, Castenholz RW (2007) Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. Environ Microbiol 9(6):1448–1455

Fleming ED, Bebout BM, Castenholz RW (2007) Effects of salinity and light intensity on the resumption of photosynthesis in rehydrated cyanobacterial mats from Baja California Sur, Mexico. J Phycol 43:15–24

Fleming ED, Castenholz RW (2008) Effects of nitrogen source on the synthesis of the UV-screening compound, scytonema, in the cyanobacterium *Nostoc punctiforme* PCC 73102. FEMS Microb Ecol 63(3):301–308

Freeman KR, Pescador MY, Reed SC, Robeson SC, Schmidt SK, Townsend AR (2009) Soil CO_2 flux and photoautotrophic community composition in high-elevation, 'barren' soil. Environ Microbiol 11(3):674–686

Friedmann EI (1982) Endolithic microorganisms in the Antarctic cold desert. Science 215:1045–1053

Friedmann EI, Kibler AP (1980) Nitrogen economy of endolithic microbial communities in hot and cold deserts. Microb Ecol 6:95–108

Friedmann EI, Ocampo-Friedmann R (1984) Endolithic microorganisms in extreme dry environments: analysis of a lithobiontic microbial habitat. In: Klug MJ, Reddy CA (eds) Current perspectives in microbiology. American Society for Microbiology, Washington, DC, pp 177–185

Friedmann EI, Ocampo-Friedmann R (1995) A primitive cyanobacterium as pioneer microorganism for terraforming Mars. Adv Space Res 15:143–246

Friedmann EI, Lipkin Y, Ocampo-Paus R (1967) Desert algae of the Negev (Israel). Phycologia 6:185–196

Fujita Y, Nakahara H (2006) Variations in the microalgal structure in paddy soil in Osaka, Japan: comparison between surface and subsurface soils. Limnology 7(2):83–91

Fukuda S, Yamakawa R, Hirai M, KashinoY KH, Satoh K (2008) Mechanisms to avoid photoinhibition in a desiccation-tolerant cyanobacterium, *Nostoc commune*. Plant Cell Physiol 49(3): 488–492

Gao KS (1998) Chinese studies on the edible blue-green alga, Nostoc flagelliforme: a review. J Appl Phycol 10:37–49

Gao KS, Ye CP (2003) Culture of the terrestrial cyanobacterium, *Nostoc flagelliforme* (Cyanophyceae), under aquatic conditions. J Phycol 39:617–623

Gao KS, Ye CP (2007) Photosynthetic insensitivity of the terrestrial cyanobacterium *Nostoc flagelliforme* to solar UV radiation while rehydrated or desiccated. J Phycol 43:628–635

Gao KS, Yu AJ (2000) Influence of CO_2, light and watering on growth of *Nostoc flagelliforme*. J Appl Phycol 12:185–189

Gao KS, Qiu BS, Xia JR, Yu AJ, Li YG (1998) Effect of wind speed on loss of water from *Nostoc flagelliforme* colonies. J Appl Phycol 10:55–58

Gao S, Ye X, Chu Y, Dong M (2010) Effects of biological crusts on profile distribution of soil water, organic carbon and total nitrogen in Mu Us Sandland, China. Plant Ecol 3(4):279–284

Garbacki N, Gloaguen V, Damas J, Hoffmann L, Tits M, Angenot L (2000) Inhibition of croton oil-induced oedema in mice ear skin by capsular polysaccharides from cyanobacteria. Naunyn Schmiedebergs Arch Pharmacol 361(4):460–464

Garcia-Pichel F, Belnap J (1996) Microenvironments and microscale productivity of cyanobacterial desert crusts. J Phycol 32:774–782

Garcia-Pichel F, Pringault O (2001) Cyanobacteria track water in desert soils. Nature 413:380–381

Garcia-Pichel F, Lopez-Cortes A, Nubel U (2001) Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. Appl Environ Microbiol 67(4):1902–1910

Geitler L (1932) Cyanophyceae. In: Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz 14. Akademische Verlagsgesellschaft, Leipzig, 1356 pp

George DB, Roundy BA, St Clair LL, Johansen JR, Schaalje GB, Webb BL (2003) The effects of microbiotic soil crusts on soil water loss. Arid Land Res Manage 17:113–125

Gundlapally SR, Garcia-Pichel F (2006) The community and phylogenetic diversity of biological soil crusts in the Colorado Plateau studied by molecular fingerprinting and intensive cultivation. Microb Ecol 52:345–357

Guo YR, Zhao HL, Zuo XA, Sam D, Zhao XY (2008) Biological soil crust development and its topsoil properties in the process of dune stabilization, Inner Mongolia, China. Environ Geol 54(3): 653–662

Gupta S, Agrawal SC (2006) Survival of blue-green and green algae under stress conditions. Folia Microbiol 51(2):121–128

Gupta S, Agrawal SC (2008) Vegetative survival of some wall and soil blue-green algae under stress conditions. Folia Microbiol 53(4):343–350

Harel Y, Ohad I, Kaplan A (2004) Activation of photosynthesis and resistance to photoinhibition in cyanobacteria within biological desert crust. Plant Physiol 136:3070–3079

Helm RF, Huang Z, Edwards D, Leeson H, Peery W, Potts M (2000) Structural characterization of the released polysaccharide of desiccation-tolerant *Nostoc commune* DRH-1. J Bacteriol 182:974–982

Higo A, Suzuki T, Ikeuchi M, Ohmori M (2007) Dynamic transcriptional changes in response to rehydration in *Anabaena* sp. PCC 7120. Microbiology 153:3685–3694

Hirai M, Yamakawa R, Nishio J, Yamaji T, Kashino Y, Koike H, Satoh K (2004) Deactivation of photosynthetic activities is triggered by loss of a small amount of water in a desiccation-tolerant cyanobacterium, *Nostoc commune*. Plant Cell Physiol 45(7):872–878

Hokputsa S, Hu CX, Paulsen BS, Harding SE (2003) A physico-chemical comparative study on extracellular carbohydrate polymers from five desert algae. Carbohydr Polym 54(1):27–32

Hong Y, Li YY, Ley SH (1992) Preliminary study on the blue-green algae community of arid soil in Qaidam Basin. Acta Bot Sin 34:161–168

Housman DC, Powers HH, Collins AD, Belnap J (2006) Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. J Arid Environ 66:620–634

Hu CX, Liu YD (2003a) Primary succession on algal community structure in desert soil. Acta Bot Sin 45(8):917–924

Hu CX, Liu YD (2003b) Vertical distribution of algae in semi-desert soil of Shapotou area Ningxia Hui Autonomous Region. Acta Ecol Sin 23:38–44

Hu CX, Liu YD (2003c) Soil algal biomass and its influential factors in desert soil crusts. Acta Ecol Sin 23(2):284–291

Hu CX, Liu YD, Song LR (1999) Species composition and distribution of algae in Shapotou area, Ningxia Hui Autonomous region, China. Acta Hydrobiol Sin 23(5):443–448

Hu CX, Liu YD, Song LR, Zhang DL (2002a) Effect of desert soil algae on the stabilization of fine sands. J Appl Phycol 14:281–292

Hu CX, Liu YD, Zhang DL, Huang ZB, Paulsen BS (2002b) Cementing mechanism of algal crusts from desert area. Chin Sci Bull 47:1361–1368

Hu CX, Liu YD, Paulsen BS, Petersen D, Klaveness D (2003a) Extracellular carbohydrate polymers from five species of soil algae with different cohesion in the stabilization of fine sand grains. Carbohydr Polym 54(1):33–42

Hu CX, Ma HY, Pang HL, Zhang DL (2003b) Species composition and distribution of algae in Wuquanshan of Lanzhou. Acta Bot Boreal Occident Sin 23(12):2099–2106

Hu CX, Zhang BC, Ma HY, Liu YD, Zhang DL (2003c) Species composition and community structure of terrestrial algae in the biological crusts of Lanzhou Northern Hill. J Northwest Norm Univ 39:59–63

Hu CX, Zhang DL, Huang ZB, Liu YD (2003d) The vertical microdistribution of cyanobacteria and green algae within desert crusts and the development of the algal crusts. Plant Soil 257: 91–111

Hu CX, Zhang DL, Liu YD (2004) Research progresses on algae of the microbial crusts in arid and semiarid regions. Prog Nat Sci 14(4): 289–295

Huang ZB, Liu YD, Smestad Paulsen B, Klaveness D (1998) Studies on polysaccharides from three edible species of *Nostoc* (cyanobacteria) with different colony morphologies: comparison of monosaccharide compositions and viscosities of polysaccharides from field colonies and suspension cultures. J Phycol 34:962–968

Ibraheem IBM (2007) Cyanobacteria as alternative biological conditioners for bioremediation of barren soil. Egypt J Phycol 8:99–116

Issa OM, Trichet J, Defarge C, Couté A, Valentin C (1999) Morphology and microstructure of microbiotic soil crusts on a tiger bush sequence (Niger, Sahel). Catena 37:175–196

Issa OM, Defarge C, Le Bissonnais Y, Marin B, Duval O, Bruand A, D'Acqui L, Nordenberg S, Annerman M (2007) Effects of the inoculation of cyanobacteria on the microstructure and the structural stability of a tropical soil. Plant Soil 290(1–2):209–219

Johnson HE, King SR, Banack SA, Webster C, Callanaupa WJ, Cox PA (2008) Cyanobacteria (*Nostoc commune*) used as a dietary item in the Peruvian highlands produce the neurotoxic amino acid BMAA. J Ethnopharmacol 118:159–165

Kang L (1999) Strategies for desertification control and ecological agricultural construction in Hexi Corridor. J Desert Res 19:195–198 (in Chinese)

Khan Z, Park SD, Shin SY, Bae SG, Yeon IK, Seo YJ (2005) Management of *Meloidogyne incognita* on tomato root-dip treatment in culture filtrate of the blue-green alga, *Microcoleus vaginatus*. Bioresour Technol 96(12):1338–1341

Kirkwood AE, Henley WJ (2006) Algal community dynamics and halotolerance in a terrestrial, hypersaline environment. J Phycol 42:537–547

Kirkwood AE, Buchheim JA, Buchheim MA, Henley WJ (2008) Cyanobacterial diversity and halotolerance in a variable hypersaline environment. Microb Ecol 55:453–465

Komárek J, Anagnostidis K (2005) Cyanoprokaryota 2. Teil/Part 2 Oscillatoriales. Spektrum. Akademischer Verlag, Heidelberg, 759 pp

Kulik MM (1995) The potential for using cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur J Plant Pathol 101:585–599

Lacap DC, Warren-Rhodes KA, McKay CP, Pointing SB (2011) Cyanobacteria and chloroflexi-dominated hypolithic colonization of quartz the hyper-arid core of the Atacama Desert, Chile. Extremophiles 15:31–38

Lalley JS, Viles HA (2005) Terricolous lichens in the northern Namib Desert of Namibia: distribution and community composition. Lichenologist 37:77–91

Lan SB, Hu CX, Rao BQ, Wu L, Zhang DL, Liu YD (2010a) Non-rainfall water sources in the topsoil and their changes during formation of man-made algal crusts at the eastern edge of Qubqi Desert, Inner Mongolia. Sci China Life Sci 53(9):1135–1141

Lan SB, Wu L, Zhang DL, Hu CX, Liu YD (2010b) Effects of drought and salt stresses on man-made cyanobacterial crusts. Eur J Soil Biol 46:381–386

Lan SB, Wu L, Zhang DL, Hu CX (2011a) Ethanol outperforms multiple solvents in the extraction of chlorophyll-*a* from biological soil crusts. Soil Biol Biochem 43:857–861

Lan SB, Wu L, Zhang DL, Hu CX (2011b) Successional stages of biological soil crusts and their microstructure variability in Shapotou region (China). Environ Earth Sci. doi:10.1007/s1 2665 -011-1066-0

Langston G, Neuman CM (2005) An experimental study on the susceptibility of crusted surfaces to wind erosion: a comparison of the strength properties of biotic and salt crusts. Geomorphology 72(1–4):40–53

Lesica P, Shelly JS (1992) Effect of cryptogamic soil crust on the population dynamics of *Arabis fecunda* (Brassicaceae). Am Midl Nat 128:58–60

Li XR, Wang XP, Li T, Zhang JG (2002) Microbiotic soil crust and its effect on vegetation and habitat on artificially stabilized desert dunes in Tengger Desert, North China. Biol Fertil Soils 35(3):147–154

Liu XJ, Chen F (2003) Cell differentiation and colony alteration of an edible terrestrial cyanobacterium *Nostoc flagelliforme*, in liquid suspension cultures. Folia Microbiol 48(5):619–626

Liu YZ, Dong GR, Li CZ (1994) A study on the factors influencing soil erosion through wind tunnel experiments. Chin J Arid Land Res 7(2):359–367

Liu YD, Shen YW, Song LR, Zhu YZ, Zhuang HR, Qin SW (1999) Species composition of algae and the relationship with fertility of soils in the middle reach of Yellow River, China. Acta Hydrobiol Sin 23(5):434–442

Liu YD, Song LR, Shen YW, Li DH, Hu CX, Huang ZB, Hu ZL, Zhu YZ (2001) Potential of terrestrial microalgae and cyanobacteria in environmental technology. In: Kojima H, Lee YK (eds) Photosynthetic microorganisms in environmental biotechnology. Springer, Hong Kong, pp 195–216, 310 pp

Liu LC, Li SZ, Duan ZH, Li XR (2006) Effects of microbiotic crusts on dew deposition in the restored vegetation area at Shapotou, northwest China. J Hydrol 328(1–2):331–337

Liu YD, Cockell CS, Wang GH, Hu CX, Chen LZ, De Philippis R (2008) Control of lunar and martian dust – experimental insights from artificial and natural cyanobacterial and algal crusts in the desert of Inner Mongolia, China. Astrobiology 8(1):75–86

Lukešová A (1993) Soil algae in four secondary successional stages on abandoned fields. Algol Stud 71:81–102

Maestre FT, Martin N, Diez B, López-Poma R, Santos F, Luque I, Cortina J (2006) Watering, fertilization, and slurry inoculation promote recovery of biological crust function in degraded soils. Microb Ecol 52:365–377

Mager DM, Thomas AD (2010) Extracellular polysaccharides from cyanobacterial soil crusts: a review of their role in dryland soil processes. J Arid Environ 72(2):91–97

Malliga P, Subramanian G (2002) Cyanobacterial biofertilizer for sustainable agriculture. In: Reddy SM, Reddy R, Sindarachary S, Girishnan S (eds) Proceedings of national symposium on bio-inoculants for sustainable agriculture and forestry. Scientific publishers, Jodhpur, India, pp 99–106

Marker AFH (1995) Chlorophyll analysis: standard methods. National Rivers Authority, Bristol

Marker AFH, Nusch EA, Rai H, Reimann H (1980) The measurement of photosynthetic pigments in freshwaters and standardization of methods, conclusions and recommendations. Arch Hydrobiol 14:91–106

Mataloni G, Tell G (2002) Microalgal communities from orthnogenic soils at Cierva Point, Antarctic Peninsula. Polar Biol 25(7): 488–491

Mataloni G, Tell G, Wynn-Williams DD (2000) Structure and diversity of soil algal communities from Cierva Point (Antarctic Peninsula). Polar Biol 23(3):205–211

Mazor G, Kidron GJ, Vonshak A, Abeliovich A (1996) The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts. FEMS Microbiol Ecol 21:121–130

McCay CP, Friedmann EI, Gomez-Silva B, Cacere L, Andersen D, Landheim R (2003) Temperature and moisture conditions in the extreme arid regions of the Atacama Desert: four years of observations including the El Nio of 1997–1998. Astrobiology 3: 393–406

McKenna-Neuman C, Maxwell CD, Boulton JW (1996) Wind transport of sand surface crusted with photoautotrophic microorganisms. Catena 27:229–247

Metting B (1981) The systematics and ecology of soil algae. Bot Rev 47:195–312

Micheletti E, Colica G, Viti C, Tamagnini P, De Philippis R (2008) Selectivity in the heavy metal removal by exopolysaccharide-producing cyanobacteria. J Appl Microbiol 105:88–94

Navarro-González R, Rainey FA, Molina P, Bagaley DR, Hollen BJ, de la Rosa J, Small AM, Quinn RC, Grunthar CL, Gomez-Silva B, McKay CP (2003) Mars-like soils in the Atacama Desert, Chile, and the dry limit for microbial life. Science 302(5647): 1018–1021

Nayak S, Prasanna R, Prasanna BM, Sahoo D (2009) Genotypic and phenotypic diversity of *Anabaena* isolates from diverse rice agro-ecologies of India. J Basic Microbiol 49(2):165–177

Nisha R, Kaushik A, Kaushik CP (2007) Effect of indigenous cyanobacterial application on structural stability and productivity of an organically poor semi-arid soil. Geoderma 138:49–56

Niyogi KK (1999) Photoprotection revisited: genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol 50:333–359

Novis PM, Smissen RD (2006) Two genetic and ecological groups of *Nostoc commune* in Victoria Land, Antarctica, revealed by AFLP analysis. Antarct Sci 18(4):573–581

Novis PM, Whitehead D, Gregorich EG, Hunt JE, Sparrow AD (2007) Annual carbon fixation in terrestrial populations of *Nostoc commune* (Cyanobacteria) from an Antarctic dry valley is driven by temperature regime. Glob Change Biol 13(6):1224–1237

Obana S, Miyamoto K, Morita S, Ohmori M (2007) Effect of *Nostoc* sp. on soil characteristics, plant growth and nutrient uptake. J Appl Phycol 19:641–646

Omoregie EO, Crumbliss LL, Bebout BM, Zehr JP (2004) Determination of nitrogen-fixing phylotypes in *Lyngbya* sp. and *Microcoleus chthonoplastes* cyanobacterial mats from Guerrero Negrao, Baja, California, Mexico. Appl Environ Microbiol 70(4):2119–2128

Page-Sharp M, Behm CA, Smith GD (1999) Involvement of the compatible solutes trehalose and sucrose in the response to salt stress of a cyanobacterial *Scytonema* species isolated from desert soils. Biochim Biophys Acta 1472:519–528

Painter TJ (1993) Carbohydrate polymers in desert reclamation the potential of microalgal biofertilizers. Carbohydr Polym 20:77–86

Pandey KD, Shukla PN, Giri DD, Kashyap AK (2005) Cyanobacteria in alkaline soil and the effect of cyanobacteria inoculation with pyrite amendments on their reclamation. Biol Fertil Soil 41:451–457

Pankratova EM (2006) Functioning of cyanobacteria in soil ecosystems. Eurasia Soil Sci 39(Suppl 1):S118–S127

Pattanaik B, Roleda MY, Schumann R, Karsten U (2002) Isolate-specific effects of amino acids and scytonemin is responsible for the UV-insensitivity of photosynthesis in *Nostoc flagelliforme*. J Photochem Photobiol B 66(1):47–53

Pardo MT, Lopez-Fando C, Almendros G, Herrero A (2009) Laboratory assessment of *Nostoc* 9v (cyanobacteria) effects on N_2 fixation and chemical fertility of degraded Arican soils. Commun Soil Sci Plant Anal 40(7–8):1295–1321

Pereira S, Zille A, Micheletti E, Moradas-Ferreira P, De Philippis R, Tamagnini P (2009) Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev 33:917–941

Pluis JLA (1994) Algal crust formation in the inland dune area Laarder Wasmeer, The Netherlands. Vegetatio 13:41–51

Pointing SB, Warren-Rhodes KA, Lacap D, Rhodes K, McKay CP (2007) Hypolithic community shifts occur as a result of liquid water availability along environmental gradients in China's hot and cold hyperarid deserts. Environ Microbiol 9:414–424

Pointing SB, Chan Y. Lacap DC, Lau MCY, Jurgens JA, Farrell RL (2009) Highly specialized microbial diversity in hyper-arid polar desert. Proc Natl Acad Sci USA 106(47):19964–19969

Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755–805

Potts M (1999) Minireview: mechanisms of desiccation tolerance in cyanobacteria. Eur J Phycol 34:319–332

Potts M (2000) Nostoc. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 465–504, 699 pp

Potts M (2001) Desiccation tolerance: a simple process? Trends Microbiol 9:553–559

Prasanna R, Saxena AK, Jaiswal P, Nayak S (2006) Development of alternative support system for viable count of cyanobacteria by most probable number method. Folia Microbiol 51(5):455–458

Pringault O, Garcia-Pichel F (2004) Hydrotaxis of cyanobacteria in desert crusts. Microb Ecol 47:366–373

Qiu BS, Gao KS (1999) Dried field populations of *Nostoc flagelliforme* (Cyanophyceae) require exogenous nutrients for their photosynthetic recovery. J Appl Phycol 11:535–541

Qiu BS, Gao KS (2002) Daily production and photosynthetic characteristics of *Nostoc flagelliforme* grown under ambient and elevated CO_2 conditions. J Appl Phycol 14:77–83

Qiu BS, Zhang AH, Liu ZL, Gao KS (2004a) Studies on the photosynthesis of the terrestrial cyanobacterium *Nostoc flagelliforme* subjected to desiccation and subsequent rehydration. Phycologia 43(5):521–528

Qiu BS, Zhang AH, Zhou WB, Wei JM, Hui D (2004b) Effects of potassium on the photosynthetic recovery of the terrestrial cyanobacterium *Nostoc flagelliforme* (Cyanophyceae) during rehydration. J Phycol 40:323–332

Rao BQ, Wang WB, Lan SB, Li DH, Hu CX, Liu YD (2009a) Development characteristics and distribution of microorganisms within 3-year-old artificial algal crust in Hopq Desert. Acta Hydrobiol Sin 33(5):937–944

Rao BQ, Wang WB, Li DH, Hu CX, Lan SB (2009b) Influence of dew on biomass and photosystem II activity of cyanobacterial crusts in the Hopq Desert, northwest China. Soil Biol Biochem 41(12):2387–2393

Rastogi RP, Sinha RP (2009) Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnol Adv 27(4):521–539

Read CF, Duncan DH, Vesk PA, Elith J (2011) Surprisingly fast recovery of biological soil crusts following livestock removal in southern Australia. J Veg Sci. doi:10.1111/j.1654-1103.2011.01296.x

Reddy PM, Roger PA, Ventura W, Watanabe I (1986) Blue-green algal treatment and inoculation had no significant effect on rice yield in an acidic wetland soil. Philipp Agric 69:629–632

Redfield E, Barns SM, Belnap J, Daane LL, Kuske CR (2002) Comparative diversity and composition of cyanobacteria in three predominant soil crusts of the Colorado Plateau. FEMS Microbiol Ecol 40(1):55–63

Rippka R, Waterbury JW, Herman M, Castenholz RW (2001) Form-genus 1. *Chroococcidiopsis* Geitler 1933, emend. Waterbury and Stanier 1978. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 528–532, 721 pp

Rivera-Aguilar V, Montjano G, Rodriguez-Zaragoza S, Duran-Diaz A (2006) Distribution and composition of cyanobacetria, mosses and lichens of the biological soil crusts of the Tehuacan Valley, Puebla, Mexico. J Arid Environ 67:208–225

Roger PA, Santiago-Ardales S, Reddy PM, Watanabe I (1987) The abundance of heterocystous blue-green algae in rice soils and inocula used for application in rice fields. Biol Fertil Soils 5:98–105

Rogers SL, Burns RG (1994) Changes in aggregate stability, nutrient status, indigenous microbial populations, and seeding emergence, following inoculation of soil *Nostoc muscorum*. Biol Fertil Soils 18:209–215

Roney BR, Renhui L, Banack SA, Murch S, Honegger R, Cox PA (2009) Consumption of *fa cai Nostoc* soup: a potential for BMAA exoisure from *Nostoc* cyanobacteria in China. Amyotroph Lateral Scler 2009(Suppl 2):44–49

Rosentreter R, Belnap J (2003) Biological soil crusts of North America. In: Belnap J, Lange OL (eds) Biological soil crusts: structure, function, and management. Springer, New York, pp 31–50

Rychert RC (2002) Assessment of cryptobiotic crust recovery. West N Am Nat 62(2):223–226

Safonova E, Reisser W (2005) Growth promoting and inhibiting effects of extracellular substances of soil microalgae and cyanobacteria on *Escherichia coli* and *Micrococcus luteus*. Phycol Res 53(3):189–193

Schlesinger WH, Pippin J, Wallenstein M, Hofmockel K, Klepeis D, Hahall B (2003) Community composition and photosynthesis by photoautotrophs under quartz pebbles, southern Mojave Desert. Ecology 84:3222–3231

Schwabe GH (1960) Blaualgen aus ariden Böden. Forsch Fortsch 34:194–197

Shachak M, Steinberger Y (1980) An algae – desert snail food chain: energy flow and soil turnover. Oecol Berl 146:402–411

Shachak M, Chapman EA, Steinberger Y (1976) Feeding, energy flow and soil turnover in the desert isopod *Hemilepistus reanmuri*. Oecologia 24:57–69

Shaw E, Hill DR, Brittain N, Wright DJ, Tauber U, Marand H, Helm RF, Potts M (2003) Unusual water flux in the extracellular polysaccharide of the cyanobacterium *Nostoc commune*. Appl Environ Microbiol 69(9):5679–5684

Shirkey B, Kovarcik DP, Wright DJ, Wilmoth G, Prickett TF, Helm RF, Gregory EM, Potts M (2000) Active Fe-containing

superoxide dismutase and abundant *sodF* mRNA in *Nostoc commune* (Cyanobacteria) after years of desiccation. J Bacteriol 182(1): 189–197

Shirkey B, McMaster NJ, Smith SC, Wright DJ, Rodriguez H, Jaruga P, Birincioglu M, Helm RF, Potts M (2003) Genomic DNA of *Nostoc commune* (Cyanobacteria) becomes covalently modified during long-term (decades) desiccation but is protected from oxidative damage and degradation. Nucleic Acids Res 31(12):2995–3005

Siegesmund MA, Johansen JR, Karsten U, Friedl T (2008) *Coleofasciculus* Gen. Nov. (Cyanobacteria): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. J Phycol 44(6):1572–1585

Singh RN (1950) Reclamation of usar lands in India through blue-green algae. Nature 165:325–326

Soule T, Stout V, Swingley WD, Meeks JC, Garcia-Pichel F (2007) Molecular genetics and genomic analysis of scytonemin biosynthesis in *Nostoc punctiforme* ATCC 29133. J Bacteriol 189:4465–4472

Soule T, Palmer K, Gao Q, Potrafka RM, Stout V, Garcia-Pichel F (2009) A comparative genomics approach to understanding the biosynthesis of the sunscreen scytonemin in cyanobacteria. BMC Genomics 10:336–345

Stal LJ (2000) Cyanobacterial mats and stromatolites. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 61–120, 669 pp

Starkenburg SR, Reitenga KG, Freitas T, Johnson S, Chain PSG, Garcia-Pichel F, Kuske CR (2011) The genome of the cyanobacterium *Microcoleus vaginatus* FGP-2, a photosynthetic ecosystem engineer of arid land soil biocrusts worldwide. J Bacteriol. doi:10.1128/JB.05138-11

Su YG, Li XR, Cheng YW, Tan HJ, Jia RL (2007) Effects of biological soil crusts on emergence of desert vascular plants in North China. Plant Ecol 191:11–19

Svircev Z, Cetojevic-Simin D, Simeunovic J, Karaman M, Stojanovic D (2008) Antibacterial, antifungal and cytotoxic activity of terrestrial cyanobacterial strains from Serbia. Sci China C Life Sci 51(10): 941–947

Swarnalakshmi K, Dhar DW, Singh PK (2007) Evaluation of blue-green algal inoculation on specific soil parameters. Acta Agron Hung 55(3):307–313

Tang D, Shi S, Li D, Hu C, Liu Y (2007a) Physiological and biochemical responses of *Scytonema javanicum* (cyanobacterium) to salt stress. J Arid Environ 71:312–320

Tang DS, Wang WB, Li DH, Hu CX, Liu YD (2007b) Effects of artificial algal crust on soil enzyme activities of Hobq Desert, China. Acta Hydrobiol Sin 31(3):339–344

Tirkey J, Adhikary SP (2005) Cyanobacteria in biological soil crusts of India. Curr Sci 89(3):515–521

Tiwari BS, Tripathi SN (1998) Effect of hydration and dehydration on initiation and dynamics of some physiological reactions in desiccation tolerant cyanobacterium *Scytonema geitleri*. Indian J Biochem Biophys 35(3):172–178

Tripathi RD, Dwivedi S, Shukla MK, Mishra S, Srivastava S, Singh P (2008) Role of blue green algae biofertilizer in ameliorating the nitrogen demand and fly-ash stress to the growth and yield of rice (*Oryza sativa* L.) plants. Chemosphere 70(10):1919–1929

Tsujimura S, Nakahara H, Kosaki T, Ishida N, Iskakov AR (1998) Distribution of soil algae in salinized irrigation land in the arid region of Central Asia – II a case study of 25-year old Bakbakty farm in the flood plain of the River Ili, Kazakstan. Soil Sci Plant Nutr 44(1):67–76

Tsujimura S, Nakahara H, Ishida N (2000) Estimation of soil algal biomass in salinized irrigation land: a comparison of culture dilution and chlorophyll a extraction methods. J Appl Phycol 12:1–8

Valentin C, Rajot JL, Mitja D (2004) Responses of soil crusting, runoff and erosion to fallowing in the sub-humid and semi-arid regions of West Africa. Agric Ecosyst Environ 104(2):287–302

Veluci RM, Neher DA, Weicht TR (2006) Nitrogen fixation and leaching of biological soil crust communities in mesic temperate soils. Microb Ecol 51(2):189–196

Vigna MS, Alberghina J, Belmonte CF (2001) Remarks about the taxonomical position of *Nodularia harveyana* Thuret ex Bornet et Flahault (Cyanophya, Nostocales) growing on soil. Nova Hedwig 72(1–2):241–250

Wang GH, Hu CX, Li DH, Zhang DL, Li XY, Chen K, Liu YD (2007a) The response of antioxidant systems in *Nostoc sphaeroides* against UV-B radiation and the protective effects of exogenous antioxidants. Adv Space Res 39(6):1034–1042

Wang WB, Yang CY, Tang DS, Li DH, Liu YD, Hu CX (2007b) Effects of sand burial on biomass, chlorophyll fluorescence and extracellular polysaccharides of man-made cyanobacterial crusts under experimental conditions. Sci China C Life Sci 50(4):530–534

Wang GH, Chen K, Chen LZ, Hu CX, Zhang DL, Liu YD (2008) The involvement of the antioxidant system in protection of desert cyanobacterium *Nostoc* sp. against UV-B radiation and the effects of exogenous antioxidants. Ecotoxicol Environ Saf 69:150–157

Wang WB, Liu YD, Li DH, Hu CX, Rao BQ (2009) Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. Soil Biol Biochem 41:926–929

Warren SD (2001) Biological soil crusts and hydrology in North American deserts. In: Belnap J, Lange OL (eds) Biological soil crusts: structure, function, and management. Springer, Berlin, pp 327–377, 503 pp

Warren-Rhodes KA, Rhodes K, Pointing S, Ewing S, Lacap D, Gómez-Silva B, Amundso R, Freidmann EI, McKay CP (2006) Hypolithic cyanobacteria, dry limit of photosynthesis and microbial ecology in the hyperarid Atacama Desert. Microb Ecol 52:389–398

Warren-Rhodes KA, Rhodes KL, Boyle LN, Pointing SB, Chen Y, Liu S, Zhuo P, McKay CP (2007a) Cyanobacterial ecology across environmental gradients and spatial scales in China's hot and cold deserts. FEMS Microbiol Ecol 61:470–482

Warren-Rhodes KA, Rhodes K, Liu S, Zhou P, McKay CP (2007b) Nanoclimate environment of cyanobacterial communities in China's hot and cold hyperarid deserts. J Geophys Res Biogeosci 112. doi:10.1029/2006/G000260

Whitton BA (2000) Soils and rice-fields. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 233–255

Whitton BA, Al-Shehri AH, Ellwood NTW, Turner BL (2005) Ecological aspects of phosphatase activity in cyanobacteria, eukaryotic algae and bryophytes. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. Commonwealth Agricultural Bureau, Wallingford, pp 205–241, 399 pp

Wierzchos J, Ascasp C, McKay CP (2006) Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. Astrobiology 6(3):15–22

Wong FK, Lacap DC, Lau MCY, DA Aitchison C, Pointing DB (2010) Hypolithic microbial community of quarzt pavement in the high-altitude tundra of central Tibet. Microb Ecol 60:730–739

Wood SA, Rueckert A, Cowan DA, Cary SC (2008) Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica. ISME J 2(3):308–320

Wright DJ, Smith SC, Joardar V, Scherer S, Jervis J, Warren A (2005) UV irradiation and desiccation modulate the three-dimensional extracellular matrix of *Nostoc commune* (Cyanobacteria). J Biol Chem 280(48):40271–40281

Wu L, Lan S(B), Zhang D, Hu C(X) (2011) Small-scale vertical distribution of algae and structure of lichen soil crusts. Microb Ecol. doi:10.1007/s00248-011-9828-5

Wynn-Williams DD (2000) Cyanobacteria in desert-life at the limit? In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 341–366, 669 pp

Xie ZM, Liu YD, Hu CX, Chen LZ, Li DH (2007) Relationships between the biomass of algal crusts in fields and their compressive strength. Soil Biol Biochem 39:567–572

Xie ZM, Liu YD, Chen LZ, Hu CX, Li DH, Shen YW (2008) The effects of different cultivation conditions on the biomass and exopolysaccharide production by *Microcoleus vaginatus* Gom. Acta Hydrobiol Sin 32(2):272–275

Xu JJ, Zhang DL, Wu GQ, Wang GH, Liu YD, Hu CX (2010) The effects of wind force on the biomass and the activity of photosynthesis of *Microcoleus vaginatus* crust. Acta Hydrobiol Sin 34(3):575–581

Yang XH, Zhang KB, Zhao YJ (2001) Microbiotic soil crust – a research forefront in desertification prone area. Acta Ecol Sin 21(3):474–480 (in Chinese)

Yan-Gui S, Xin-Rong L, Ying-Wu C, Zhi-Shan Z, Yan L (2011) Carbon fixation of cyanobacterial-algal crusts after desert fixation and its implication to soil organic carbon accumulation in desert. Land Degrad Dev 22. doi:10:1002/ldr.1131

Ye C, Gao K, Giordano M (2008) The odd behaviour of carbonic anhydrase in the terrestrial cyanobacterium *Nostoc flagelliforme* during hydration-dehydration cycles. Environ Microbiol 10(4): 1018–1023

Yeager CM, Kornosky JL, Housman DC, Grote EE, Belnap J, Kuske CR (2004) Diazotrophic community structure and function in two successional stages of biological soil crusts from the Colorado plateau and Chihuahuan desert. Appl Environ Microbiol 70(2):973–983

Yoshida T, Sakamoto T (2009) Water-stress induced trehalose accumulation and control of trehalase in the cyanobacterium *Nostoc punctiforme* IAM M-15. J Gen Appl Microbiol 55(2):135–145

Yoshimura H, Okamoto S, Tsumuraya Y, Ohmori M (2007) Group 3 sigma factor gene, sigJ, a key regulator of desiccation tolerance, regulates the synthesis of extracellular polysaccharide in cyanobacterium *Anabaena* sp strain PCC 7120. DNA Res 14(1):13–24

Zaady E, Kuhn U, Wilske B, Sandoval-Soto L, Kesselmeier J (2000) Patterns of CO_2 exchange in biological soil crusts of successional age. Soil Biol Biochem 32:959–966

Zaady E, Bouskila A (2002) Lizard burrows association with successional stages of biological soil crusts in an arid sandy region. J Arid Environ 50(2):235–246

Zancan S, Trevisan R, Paoletti MG (2006) Soil algae composition under different agro-ecosystems in North-Eastern Italy. Agric Ecosyst Environ 112(1):1–12

Zhang YM, Wang HL, Wang XQ, Yang WK, Zhang DY (2006) The microstructure of microbiotic crust and its influence on wind erosion for a sandy soil surface in the Gurbantunggut Desert of Northwestern China. Geoderma 132:441–449

Zhang H, Ma HY, Zhang DL LVY, Wang GH, Chen K, Liu YD, Hu CX (2008) On apoptosis of human epidermoid carcinoma A431 cells induced by the extracellular polymeric substances of *Scytonema javanicum*. Acta Hydrobiol Sin 32(6):89–95

Zhang BC, Zhang YM, Downing A, Niu Y (2011) Distribution and composition of cyanobacteria and microalgae associated with biological soil crusts in the Gurbantunggut Desert, China. Arid Land Res Dev 25(3):275–293

Zhao J, Zheng Y, Zhang B, Chen Y, Zhang Y (2009) Progress in the study of algae and mosses in biological soil crusts. Front Biol China 4(2):143–150. doi:10.1007/(5)1515-008-0104-0

Cyanobacteria in High Latitude Lakes, Rivers and Seas

13

Warwick F. Vincent and Antonio Quesada

Contents

Summary

Cyanobacteria often account for a large and sometimes dominant fraction of phototrophic biomass and primary production in high latitude lakes, ponds, streams and wetlands. Picocyanobacteria are usually the most abundant photosynthetic cell type in the plankton of Arctic lakes and rivers, and in East Antarctic saline lakes they have been recorded at cell concentrations of up to 1.5×10^7 per mL. In striking contrast to their success in high latitude lakes, picocyanobacteria are generally absent or sparse in polar seas, with the exception of regions that receive advective inputs of picocyanobacteria from more favourable growth environments. Colonial bloom-forming cyanobacteria are conspicuously absent from most polar freshwaters, but future climate change may favour their development in some areas via warmer temperatures for growth, more stable water columns that favour gas-vacuolate species and richer nutrient conditions as a result of more active catchment processes. Mat-forming cyanobacteria are a ubiquitous element of polar aquatic ecosystems including lakes, ponds, streams and seeps. These consortia of diverse microbial taxa often occur as benthic crusts and films, and in some locations form luxuriant communities up to tens of cm in thickness. They have many biological features that make them well suited to life in the extreme polar environment, including tolerance of persistent low temperatures, freeze-thaw-cycles, high and low irradiances, UV-exposure and desiccation.

13.1 Introduction

The first accounts of cyanobacteria in the polar regions came from reports by explorers who noticed unusual biological communities growing on or beneath the ice. In the expedition sent by Robert Falcon Scott to explore the McMurdo Dry Valleys, Antarctica in 1909, Griffith Taylor wrote: "*We came across a lake two miles long. It was of course frozen, but*

W.F. Vincent (✉)
Departement de Biologie, Université Laval,
Quebec City, QC, G1V OA6, Canada
e-mail: warwick.vincent@bio.ulaval.ca

A. Quesada
Departamento de Biologia, Facultad de Ciencias,
Universidad Autonoma de Madrid,
28049, Madrid, Spain
e-mail: antonio.quesada@uam.es

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beneath the ice the water was very deep and we could see extensive water plants" (Taylor 1916). These lakes have no aquatic macrophytes, but they do contain luxuriant, brightly coloured communities of cyanobacterial mats. Several decades earlier on his epic traverse of the Greenland Ice Cap, Nordenskiöld noted that the botanist on his expedition, Dr. Beggren, "*soon discovered, partly on the surface of the ice, partly in the above mentioned powder (cryoconite rock dust), a brown polycellular alga*" (Leslie 1879). This was later identified as the cryoconite community dominated by the nitrogen-fixing cyanobacterium *Calothrix parietina* (Gerdel and Drouet 1960). James Murray, the biologist on Shackleton's 1907–1909 expedition to Ross Island Antarctica, dug through the ice to the bottom of a frozen lake and found a benthic mat "*that on careful thawing released a multitude of living things for study*" (Murray 1910), probably the first evidence that cyanobacterial mats are refugia for diverse communities of organisms in the polar environment.

Over the last few decades there have been many advances in understanding of high latitude lakes, ponds and flowing waters, and of the composition and functioning of their biological communities. It is now well established that cyanobacteria are a major and often dominant component of polar aquatic ecosystems, with strong similarities in community types and ecology between the Arctic and Antarctica. Ecologically, most aquatic cyanobacteria fall into three functional groups (Vincent 2009): picocyanobacteria, bloom-formers, and mat-formers. The first of these groups, picocyanobacteria, are widespread in the oligotrophic freshwaters that characterize high latitude regions, and in some locations these smallest of phototrophs achieve extremely high concentrations. In contrast, there is an intriguing absence or sparse representation of picocyanobacteria in the adjacent polar oceans. Bloom-forming cyanobacteria are currently absent from most polar aquatic environments, but they have been observed in subarctic waters and may be increasingly prevalent with ongoing environmental change in the polar regions. Mat-forming species are the most successful cyanobacteria at high latitudes. These achieve spectacular biomass levels at some sites, although they are more commonly present as mm-thick mats, films and aggregates, and can also be associated with aquatic mosses (Vincent 2000; Singh and Elster 2007; Nakai et al. 2012).

In this review, we examine each of the ecological groups of cyanobacteria in the polar regions, with emphasis on high latitude lake, pond, river and stream communities. In a companion chapter (Chap. 14) we examine cyanobacterial diversity and function in more extreme habitats of the cryosphere: snow, ice, rock and soils. Many of the habitats and communities described below are illustrated in the online article associated with this book chapter (Vincent and Quesada: Cyanobacterial diversity and dominance in polar aquatic ecosystems).

13.2 Picocyanobacteria

Applications of fluorescence microscopy, flow cytometry and molecular methods have resulted in a rapid expansion in knowledge about picocyanobacteria in aquatic ecosystems (see Chaps. 8 and 20). Their high surface-to-volume ratio makes them especially well suited to life in oligotrophic waters. Given the low nutrient status of most high latitude lakes and rivers, picocyanobacteria are widely distributed and abundant in the aquatic ecosystems of both polar regions, as expected. However, they are conspicuously absent or rare in polar seas, yet these may also be nutrient-poor in some locations. *Prochlorococcus marinus*, which is highly successful in the tropical ocean, declines sharply in cell concentration at about latitudes 45°N and 45°S and there have been no confirmed reports of its occurrence in the Arctic Ocean. After extensive surveys of the Arctic picoplankton by flow cytometry, Li (2009) concluded that this taxon "*is known and confirmed to be absent from subpolar and polar waters*". No freshwater representatives of *Prochlorococcus* or the filamentous *Prochlorothrix* are known from the Arctic or Antarctica.

The picocyanobacterial genus *Synechococcus* is well represented in polar freshwaters as well as in Arctic and Antarctic saline lakes (Table 13.1), but in the ocean it steadily declines with increasing latitude south and north (Fig. 13.1), and is typically sparse or undetectable in subpolar and polar marine environments. In a 2,780-km north–south transect during winter into the Southern Ocean (Doolittle et al. 2008), phycoerythrin-rich picocyanobacteria were below the limits of detection once temperatures dropped below 1.3°C, south of the Antarctic Polar Front. *Prochlorococcus* was only detected north of the Subtropical Convergence, in water temperatures above 10°C. In the most extensive survey of picoplankton in the Arctic to date, from the Pacific Ocean through the Canadian Arctic Archipelago to the Atlantic Ocean, there was a striking increase in picocyanobacteria at either end of the transect, and the Arctic Ocean was almost devoid of picocyanobacteria (Fig. 13.2). This dichotomy of abundant *Synechococcus* in lakes yet near absence in the polar oceans has been attributed to the inhibitory effect of extreme low temperatures in the polar marine environment on cyanobacterial growth rates, which prevents them keeping pace with loss processes such as grazing, viral lysis and advection (Vincent 2000). Picoeukaryotes do not exhibit the same latitudinal decline as picocyanobacteria and are sometimes the dominant phytoplankton in the polar oceans. In the Southern Ocean transect, picoeukaryotes represented more than 99% of the picophytoplankton community in waters cooler than 1°C (Doolittle et al. 2008). An especially common picoeukaryote in the Arctic Ocean is a genetically distinct prasinophyte of the genus *Micromonas*. This genotype is psychrophilic and has relatively fast growth rates at low

Table 13.1 Concentrations of picocyanobacteria in high latitude waters.

Aquatic environment and site	Concentration range (cells mL^{-1})	References
Marine		
AO, east–west transect across Canada	0–10	Li (2009)
Greenland Sea	nd-5×10^3	Gradinger and Lenz (1995)
Coastal AO, eastern Beaufort Sea	$0.2–7\times10^4$	Waleron et al. (2007)
Coastal AO, western Beaufort Sea	$10–10^2$	Cottrell and Kirchman (2009)
Saline lakes		
Meromictic lakes, CHA	$10^3–6\times10^4$	Van Hove et al. (2008)
Vestfold Hills lakes, Antarctica	$10^4–1.5\times10^7$	Powell et al. (2005)
Freshwater lakes		
Freshwater lakes, Vestfold Hills	nd	Powell et al. (2005)
Canadian subarctic lake	$0.2–7\times10^4$	Rae and Vincent (1998)
Byers Peninsula, MA	$10^2–10^4$	Toro et al. (2007)
Eutrophic lakes, Signy Island, MA	$10^4–10^8$	Hawes (1990)
Rivers		
Great Whale River, Canadian subarctic	$1.6–4\times10^4$	Rae and Vincent (1998)
Mackenzie River, Canadian Arctic	$2–5\times10^4$	Vallières et al. (2008)

CHA Canadian High Arctic, *MA* maritime Antarctica, *AO* Arctic Ocean, *nd* not detectable

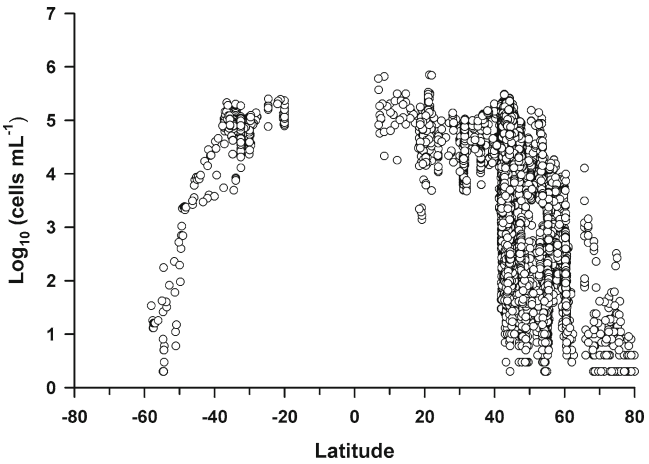

Fig. 13.1 Latitudinal trends in picocyanobacteria (*Synechococcus* plus *Prochlorococcus*), from latitudes 60°S to 80°N. (This figure was prepared by WKW Li based on data published in Li 2009)

temperature (Lovejoy et al. 2007), which may explain its striking success relative to *Synechococcus*. Future changes in the temperature regime of the Arctic Ocean as a result of global warming could ultimately lead to a shift from picoeukaryotes to picocyanobacteria, with implications for food quality and trophic processes (Vincent 2010).

Picocyanobacteria have been recorded at a few sites in the polar oceans, notably in places that have inputs of picocyanobacteria from elsewhere, for example the Beaufort Sea influenced by the Mackenzie River (Waleron et al. 2007) and the Greenland Sea influenced by advection from the North Atlantic (Gradinger and Lenz 1995). The latter study concluded that northward flowing Atlantic water in spring brings high concentrations of picocyanobacteria into the Greenland Sea, which is initially devoid of these cells, and that the presence of picocyanobacteria in this region is the result of advection from the south combined with the high survival potential of these organisms once below the threshold of grazing pressure. *Synechococcus* cells have been recorded in coastal waters of the Arctic Ocean off Barrow, Alaska. Cell concentrations were similar in summer and winter (up to 10^2 cells mL^{-1}), leading Cottrell and Kirchman (2009) to suggest that their growth in the dark may be supported by heterotrophy. These populations might also be the result of advection via coastal currents of Pacific Ocean water, combined with the high survivability of cyanobacterial cells, as in the Greenland Sea.

Studies of lakes in subarctic Québec have shown that picocyanobacteria represent 30–60% of the planktonic biomass, as measured by chlorophyll a (Bergeron and Vincent 1997), and their photosynthetic activity may be more resistant to UV radiation than for larger phytoplankton cells (Laurion and Vincent 1998). Cell concentrations of *Synechococcus* vary greatly in polar lakes, from undetectable in some freshwaters, to 1.5×10^7 cells mL^{-1} in Ace Lake and other saline lakes of the Vestfold Hills in east Antarctica (Table 13.1). In the Vestfold Hills lakes, peak populations were observed deep within the water column where nutrient concentrations are higher (Powell et al. 2005). In an analogous meromictic lake in the High Arctic, deep mixing during an unusual period of ice out was accompanied by a threefold increase in picocyanobacteria, with peak concentrations in the deeper, more phosphorus-rich waters (Veillette et al. 2011). Detectable populations of picoplanktonic cyanobacteria were also observed in the deep chlorophyll maximum of Lake Vanda in the McMurdo Dry Valleys of Antarctica (Vincent and Vincent 1982).

Picocyanobacteria are also a common constituent of high latitude rivers fed by lakes or where the flowing water transit time is long enough to allow the development of a phytoplankton community. In the Great Whale River, subarctic Canada, picocyanobacteria achieved concentrations up to

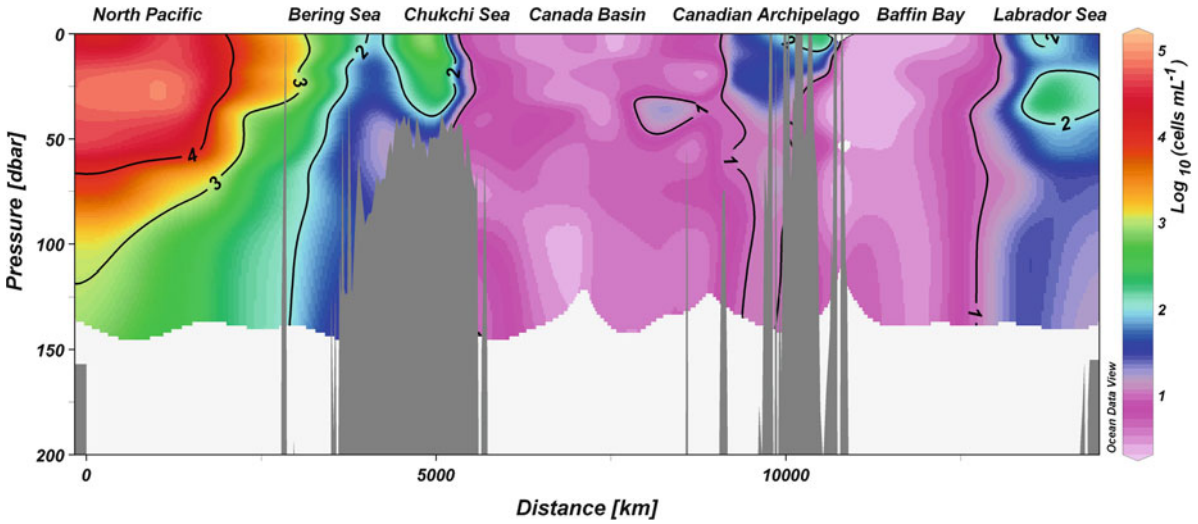

Fig. 13.2 The Arctic as a picocyanobacterial void in the World Ocean. This flow cytometry transect extended eastwards from the Pacific Ocean through the Arctic Ocean to the Labrador Sea at the edge of the Atlantic Ocean. (By permission of WKW Li and C Lovejoy [International Polar Year Symposium, Oslo, Norway, June 2010])

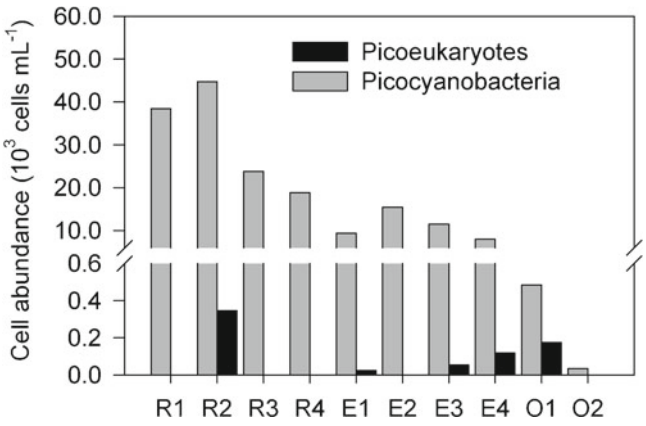

Fig. 13.3 Abundance of picocyanobacteria along a transect in the Mackenzie River, Canadian Arctic. *R1–R4* were freshwater stations in the river, *R5* and *R7* were located across the estuarine transition from freshwater to saltwater, and *R8* and *R9* in the coastal ocean. (Redrawn from the data in Vallières et al. 2008)

10^4 mL^{-1} and the picoplankton fraction (<2 μm) contributed about half of the chlorophyll *a* biomass (Rae and Vincent 1998). A 300-km transect down the Mackenzie River, in Canada's Northwest Territories, showed that picocyanobacterial concentrations ranged up to 5×10^4 cells mL^{-1}, but dropped precipitously across the estuary to around 30 cells mL^{-1} in the offshore ocean (Fig. 13.3). In the freshwater river sites, many of the picocyanobacteria occurred in cell aggregates (Vallières et al. 2008).

Initial analyses of the pigment characteristics of *Synechococcus* isolates from High Arctic lakes implied a high level of genetic diversity (Vézina and Vincent 1997). Molecular characterization of assemblages from northern Ellesmere Island similarly indicates considerable diversity among northern strains (Van Hove et al. 2008). The 16S rRNA gene clone library analysis of picocyanobacteria in the Beaufort Sea off the mouth of the Mackenzie River showed that most were closely related to freshwater and brackish *Synechococcus*. No typically marine *Synechococcus* sequences nor any *Prochlorococcus* sequences were recovered, consistent with the hypothesis that most of the picocyanobacteria in this coastal region of the Arctic Ocean come from the river (Waleron et al. 2007).

Thin trichome oscillatorians are often reported in Antarctic lakes plankton; for example, oscillatorian cyanobacteria in the bacterioplankton were found in five out of six lakes in maritime Antarctica (Schiaffino et al. 2009). These organisms also contribute to the deep chlorophyll maxima in McMurdo Dry Valley lakes (e.g. Vincent and Vincent 1982; Spaulding et al. 1994). Studies on the light-capturing abilities of thin oscillatorians have shown that these approach the efficiencies of picocyanobacteria (S. Vézina and Vincent, unpublished data). Some of these populations may be the result of resuspension from benthic mats that often grow over polar stream beds and the lake littoral zone (see below).

13.3 Bloom-Forming Cyanobacteria

Bloom-forming cyanobacterial taxa such as *Anabaena*, *Microcystis* and *Aphanizomenon* are largely absent from the polar regions. A detailed phytoplankton analysis of two lakes in the Canadian High Arctic, ultraoligotrophic Char Lake and sewage polluted Meretta Lake, showed that cyanobacteria made only a small contribution to total nano- and micro- phytoplankton biomass (<10%) in both lakes and that, unlike temperate lakes, there was no increase in the proportional

representation of this group in the enriched system (Kalff et al. 1975). Similarly, in a mesocosm experiment conducted in Char Lake, nutrient enrichment (+ P and to a greater extent, +NP) caused an increase in algal biomass, but there was no shift towards cyanobacteria, unlike the usual phytoplankton community response to nutrients in warmer lakes (Schindler 1974). These results imply that bloom-formers are absent not only as a result of the low nutrient status of most waters, but probably also because of other factors, such as low temperatures. Some bloom-formers are known to grow at cool temperatures, at least in culture (e.g. *Aphanizomenon flos-aquae*; Mehnert et al. 2010). However, in temperate latitudes blooms become more likely as the water column warms above 15°C, in part because bloom-forming cyanobacteria tend to have high temperature optima for maximum growth. This temperature-correlated effect may also be the result of an increased frequency and strength of diurnal thermoclines (near-surface temperature and density gradients), which accompany warming and potentially favour gas vacuolate species that can adjust their position in a stable water column (Vincent 2009).

The Arctic, and also maritime Antarctica, are heating rapidly as a result of global climate change, and polar microbial ecosystems are beginning to show the effects of this warming in Antarctica (Quayle et al. 2002) and the Arctic (Vincent 2010). In the longer term, high latitude lakes may become more conducive to bloom-forming cyanobacteria as a result of warmer waters, and increased nutrient input from catchments. Limnological observations at Saqvaqjuac (lat. 63°N), on the western side of Hudson Bay, Canada, are informative in this regard (Welch et al. 1989). Whole lake fertilization experiments in three of the lakes of this coastal region resulted in a strong increase in the concentration of chrysophytes, cryptophytes, dinoflagellates and green algae. As in Char Lake, colonial cyanobacteria (including *Gomphosphaeria*, *Aphanothece*, *Aphanocapsa* and *Anabaena*) were only minor constituents of the phytoplankton and did not respond to the fertilization, even under low N:P ratios. However, naturally enriched lakes close to the sea had blooms of cyanobacteria (*Anabaena* and *Oscillatoria*). This indicates the potential for such communities to develop in the North, although the controlling mechanisms are still unclear.

13.4 Mat-Forming Species

The most conspicuous cyanobacterial communities in the polar regions are mats, films and crusts over the bottom substrata of lakes, ponds, streams and other water-containing ecosystems. These are often highly pigmented, and can form mucilaginous layers, typically 0.5–5 mm thick, but sometimes much thicker (see below). In many aquatic ecosystems of the Arctic and Antarctic, these benthic communities dominate total biomass as well as total primary productivity.

The cyanobacterial-based mat communities are consortia of diverse taxa that are interdependent via their trophic and biogeochemical relationships. The assemblage is often based on a well-developed matrix of thin, filamentous cyanobacteria and a cohort of other autotrophic organisms, which collectively support short trophic chains to bacteria, protists and simple metazoans such as rotifers, nematodes and tardigrades. The chemical and physical micro-environments within the mats differ greatly from bulk properties of their surroundings, and are influenced by the local climate, water, ice and sediment characteristics as well as by the physical and biological organization of the community. Microbial mats can be considered self-organized ecosystems in which biological processes take place under much more benign conditions than in the surrounding polar environment.

13.4.1 Community Structure

Four types of benthic communities commonly occur in polar freshwaters. Firstly, the rocky substrata of shallow streambeds and ponds may be coated by black or brown coloured crusts. These are typically dominated by *Gloeocapsa*, *Schizothrix* or *Calothrix*, and are often rich in UV-screening pigments such as scytonemin (Proteau et al. 1993) or the related compound gloeocapsin. Secondly, tundra ponds often contain extensive black, dark yellow or olive-green sheets of *Nostoc commune*, which sometimes detach and float to the surface (Vincent 2000). This nitrogen-fixing species also occurs over the bottom or edge of some Antarctic stream beds. A third type of community is formed by loose spherical colonies of *Nostoc*; e.g. in meltwater pools on the McMurdo Ice Shelf, Antarctica, and in Two Basin Lake, Canadian High Arctic (Quesada et al. 1999). The fourth community, and by far the most common, is composed of benthic films and mats dominated by filamentous, mucilage-producing Oscillatoriales (Fig. 13.4). These organisms are responsible for the three-dimensional structure of the communities, and the most common genera are *Leptolyngbya*, *Phormidium*, *Microcoleus* and *Oscillatoria*.

Even within a single localized area of the polar regions there can be a large biodiversity of cyanobacteria. For example, on James Ross Island, Antarctica, 75 cyanobacterial morphotypes were observed in various habitats of the area (Komárek and Elster 2008; Komárek et al. 2008), including several types of monospecific communities. Almost all the coccoid morphotypes (in particular a large *Chroococcus*) were restricted to seepages, and were never the taxonomic dominants in the communities. The most commonly represented genus was *Leptolyngbya,* with different species in different habitats *(e.g. L. fritschiana, L. vincentii and L. borchgrevinkii). Phormidium* was also widely distributed; *Phormidium priestleyi* was found in downstream flowing waters, while *P. autumnale* was common in streams and

Fig. 13.4 A carotenoid-rich microbial mat in Discovery Pond at latitude 83°N, on the northern coast of Ellesmere Island in the Canadian High Arctic. The mat community is dominated by oscillatorian cyanobacteria embedded within a mucilaginous matrix of exopolymeric substances. (Photo WF Vincent)

seepages (Komárek et al. 2008). This latter taxon is important throughout Antarctica, and appears to be an isolated clade within the traditional genus *Phormidium*. On the basis of its distinctive morphological and molecular characteristics, it shows a greater affinity to the genus *Microcoleus* (Strunecký et al. 2010).

The application of molecular methods is showing that the cyanobacterial diversity of the polar regions is very much greater than previously thought. Even within the same apparent morphospecies there may be considerable genetic variation. For example, amplified fragment length polymorphism (AFLP) analysis of variation within *Nostoc commune* from collections in Victoria Land, Antarctica, showed that samples could be split according to habitat (irrigated soil communities versus ponds), rather than latitude (Novis and Smissen 2006).

Studies of Antarctic oscillatorians based on morphological and molecular methods have reported endemic as well as cosmopolitan taxa (Taton et al. 2003, 2006a, b; Jungblut et al. 2005; Comte et al. 2007; Komárek et al. 2008). In the most comprehensive analyses to date on microbial mat diversity in the Antarctic, Taton et al. (2003, 2006a, b) analyzed mats from Lake Fryxell (Dry Valleys), four coastal lakes in the Prydz Bay area (East Antarctica) and two meltwater samples from Livingston Island (Antarctic Peninsula). Using clone libraries based on 16S rRNA gene sequences, a total of 63 operational taxonomic units (OTUs) were defined, of which 44 were unique to Antarctica (70%) at the time of publication. In ponds at the far southern Dufek Massif (82ºS), cyanobacterial diversity was impoverished, but the sequenced clones seemed to be more closely related to clones from other Antarctic regions than to sequences from non-Antarctic regions (Fernández-Carazo et al. 2011). Conversely, a clone library analysis of oscillatorian mats at the northern limit of the High Arctic found several High Arctic ribotypes that were >99% similar to Antarctic and alpine sequences. These results included close matches to taxa that had been previously considered endemic to Antarctica, for example one sequence that was 99.8% similar to *Leptolyngbya antarctica* sequenced from the Larsemann Hills, Antarctica. More than 68% of all identified ribotypes at each High Arctic site matched only cyanobacterial sequences from perennially cold ecosystems, and these were <97.5% similar to sequences from warmer environments. These results imply the global distribution of low-temperature cyanobacterial ecotypes throughout the cold terrestrial biosphere (Jungblut et al. 2010). Similar results were obtained by Comte et al. (2007) and Michaud et al. (2012), with some genotypes showing a bipolar distribution. The restriction of endemic cyanobacteria to within one or both polar regions is becoming progressively less apparent. For example, the taxon *Phormidium murrayi,* previously considered to be endemic to Antarctica, has been found in New Zealand streams (Heath et al. 2010), and *Phormidium* sp. from Lake Fryxell has been found to be genetically highly similar (96–99%) to a strain present in saline lakes on the Chilean Altiplano (Dorador et al. 2008). However, most of these studies are based on a limited number of genes, and more detailed genomic analysis is required to fully assess the important question of endemicity versus cosmopolitanism.

Like microbial mats elsewhere, polar cyanobacterial mat and film communities are complex consortia containing viruses, heterotrophic bacteria, archaea and microbial eukaryotes. For example, analysis of 16S rRNA genes from mats collected from the moat of Lake Fryxell in the McMurdo Dry Valleys showed a high bacterial diversity, including the gliding bacteria *Stigmatella, Myxococcus, Cytophaga, Flavobacterium, Marinilabilia* and *Flexibacter*, and anaerobic saccharolytic taxa such as *Clostridium* and *Eubacterium*. Only two distinct archaeal clone sequences were recovered (Brambilla et al. 2001). Similarly, in high arctic microbial

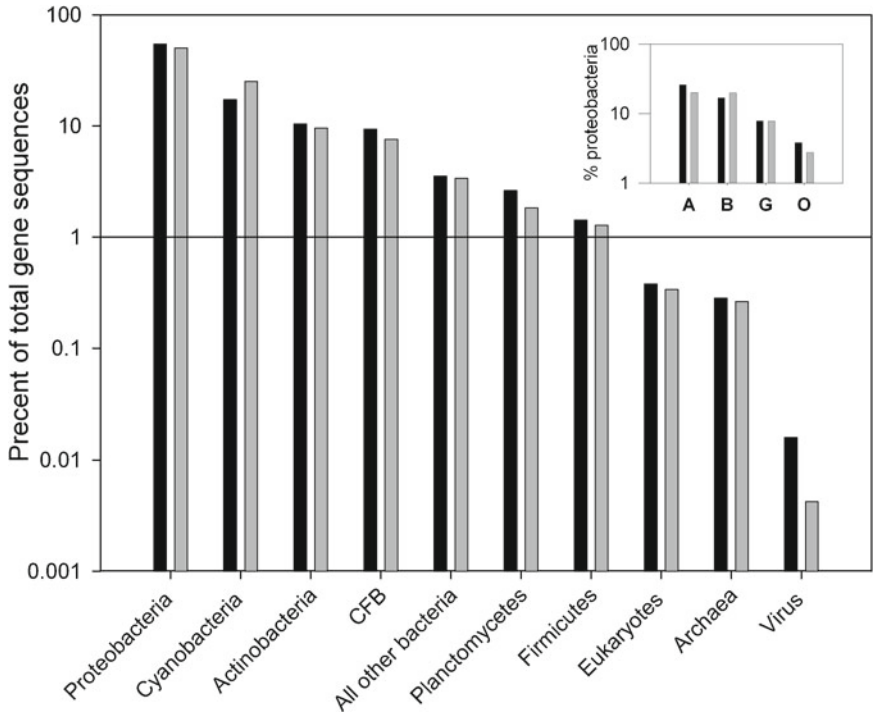

Fig. 13.5 Microbial biodiversity in polar cyanobacterial mats. This shows the distribution of protein-coding gene sequences according to major taxonomic groups, based on metagenomic analysis of mat communities from the Ward Hunt Ice Shelf (*black bars*) and Markham Ice Shelf (*grey bars*) in the High Arctic. Insert: contribution of the major classes of Proteobacteria (*A* Alphaproteobacteria, *B* Betaproteobacteria, *G* Gammaproteobacteria, *O* other Proteobacteria). Note the log scale of the *y-axis* in both graphs. (Modified from Varin et al. 2010)

mats bacterial diversity was high, but only one clade of Archaea was recovered (Bottos et al. 2008). Analyses of cryoconite hole mats on McMurdo Dry Valley glaciers that lie upstream from the Dry Valley lakes had representatives from eight bacterial lineages (*Acidobacterium, Actinobacteria,* Cyanobacteria*,* Cytophagales*, Gemmimonas, Planctomycetes, Proteobacteria,* and Verrucomicrobia), metazoa (nematode, tardigrade, and rotifer), *Choiromyces*, a ciliate (*Spathidium*) and green algae (Christner et al. 2003), and had similarities with lake ice consortia (Priscu et al. 1998) and microbial mats from Lake Fryxell. More recent studies have shown a high percentage of *Cytophaga*-flavobacteria cells in cryoconite sediments (Foreman et al. 2007). Microbial eukaryotes are often conspicuous elements of polar microbial mats (e.g., on the Markham Ice Shelf; Vincent et al. 2004a, b), and constituted ca. 20% of the protein-coding genes in a metagenomic study of Arctic cyanobacterial mats (Varin et al. 2010). This latter study also showed that the ribosomal and protein-coding genes from these mats were dominated by Proteobacteria, not Cyanobacteria, with a small contribution by Archaea and viruses (Fig. 13.5). Fungi are also present in Antarctic microbial mats (Verleyen et al. 2010) and may play an important functional role in mat decomposition processes.

The rich microbial biodiversity of polar cyanobacterial mats has generated interest for bioprospection, with the possibility that these consortia may contain microbes that produce molecules of biomedical or biotechnological potential. Some of the isolates and communities produce unusual lipids (e.g. Rezanka et al. 2009) and toxins (e.g. Hitzfeld et al. 2000; Wood et al. 2008), and a variety of antimicrobials have been identified from cyanobacterial isolates (e.g. Taton et al. 2006a; Asthana et al. 2009). The potential of Antarctica cyanobacteria for commercial production of carotenoids (Shukla and Kashyap 2003), phycocyanin (Shukla et al. 2008) and UV-screening scytonemin (Singh et al. 2010) has also been explored.

13.4.2 Mat Structure and Pigments

Microbial mats consist of diverse microbiota embedded within a polymeric gel matrix. In the polar regions, the main structural elements of the mats are usually filamentous cyanobacteria, which excrete exopolymeric substances (EPS) that bind together the assemblage. The application of novel methods in electron and confocal microscopy has produced many insights into the architecture of these assemblages, and has shown that these biofilms have an anastomosing network of holes and channels (de los Ríos et al. 2004; Fig. 13.6), which may provide conduits for nutrient transfer as well as microhabitats for smaller cells such as bacteria and even channels for the movement of gliding, filamentous cyanobacteria (Vincent and Quesada 1994).

One of the most striking features of polar cyanobacterial mats is their colouration and pigment content, to the extent that the biopotential of some high latitude isolates has been considered for commercial pigment production (Shukla and Kashyap 2003; Shukla et al. 2008). The cyanobacterial pigments include both light-harvesting and photoprotective

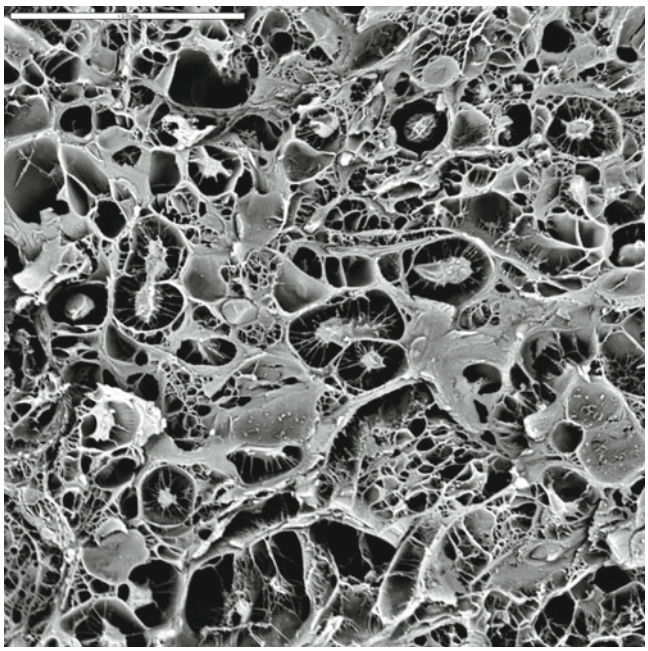

Fig. 13.6 Freeze-fracture electron micrograph of a microbial mat from the High Arctic showing its open, pore-containing structure. The scale bar is 10 μm. The scale bar is 10 μm. (A. de los Rios, with permission)

molecules, and typically dominate the total ecosystem pigment stocks. For example, in shallow ponds and lakes from Alaska, High Arctic Canada and Low Arctic (SubArctic) Canada, the chlorophyll *a* and carotenoid content of the mats (mg m^{-2}) was more than 100 times that of the phytoplankton in the overlying water column (Bonilla et al. 2009). The mats vary greatly in colour depending on species composition and light regime. Some are dull green or blue-green in colour, for example some *Nostoc* colonies and communities of oscillatorians that have migrated to the surface of mats under dim light conditions (e.g. Salt Pond on the McMurdo Ice Shelf). This coloration is dictated by the high concentrations of light-capturing phycobiliproteins in the cells, particularly phycocyanin, sometimes combined with phycoerythrin giving the communities a darker appearance. *Nostoc commune* mats that are exposed to bright light, for example in shallow Arctic tundra ponds (Vincent 2000) or in Antarctic stream beds, are often golden-brown, olive-green or black in appearance as a result of high concentrations of the UV-screening pigment scytonemin (Proteau et al. 1993). Mats and crusts of *Calothrix* (e.g., Antarctic stream bed communities and the Greenland ice cap cryoconite assemblages) are also black pigmented with scytonemin (e.g., Gerdel and Drouet 1960; Vincent 1988).

The luxuriant oscillatorian mat communities and living stromatolites at the bottom of Antarctic ice-capped lakes (e.g. McMurdo Dry Valleys lakes; Lake Untersee, Andersen et al. 2011) are often pink in colour as a result of high concentrations of the light-capturing pigment phycoerythrin and the absence of UV blocking pigments. Five types of microbial mats were initially identified in Antarctic ice-capped lakes (Wharton et al. 1983; Parker and Wharton 1985): (i) Moat mats that occur around the edge of the lake where the ice melts each summer; (ii) Lift-off mats that trap bubbles of nitrogen and oxygen in upright columnar structures up to 1 cm in diameter and that may eventually detach from the sediments; (iii) Pinnacle mats that form small cone–shaped structures; (iv) aerobic prostrate mats; (v) anaerobic prostrate mats. Larger mat structures have been subsequently discovered in some Antarctic lakes (illustrated in the online article associated with this book), including microbialites (macroscopic sedimentary structures derived from microbial growth) in Lake Joyce (Wharton et al. 1983), dome-shaped structures in Untersee (Andersen et al. 2011) and cyanobacteria-coated moss pillars in freshwater lakes near Syowa Station (Imura et al. 1999; Nakai et al. 2012).

The most commonly observed oscillatorian mat communities throughout both polar regions are in shallow waters, and are often orange, pink or purple as a result of carotenoids (Fernández-Valiente et al. 2007) that protect against the damaging effects of bright PAR and UV radiation (see below). Many of these have similar morphologies and detachment characteristics to the moat and lift-off mat categories as described in the McMurdo Dry Valley lakes; e.g., the microbial mat communities of freshwater lakes in the Hope Bay region of the Antarctic Peninsula, which vary greatly in texture, colour and thickness depending on community structure, substrate and sediment content (Bonaventura et al. 2006). The mats usually have a layered structure, with the surface enriched in photoprotective pigments and the basal layer enriched in light capturing pigments, including phycocyanin and chlorophyll a (Vincent et al. 1993; Quesada and Vincent 1997; Mueller et al. 2005). Most of the active cyanobacterial biomass resides in this 'deep chlorophyll maximum', where the cells grow under a shade regime of dim orange light that is devoid of blue and UV wavebands (Vincent et al. 1993). There are now many published reports of these brightly coloured, layered communities from both polar regions, and here we provide two such examples.

The shallow waters of Ward Hunt Lake at the northern limit of High Arctic Canada contain extensive mats that average about 4 mm in thickness. Vertical sectioning of these mats shows that they are composed of a black layer irregularly distributed over the surface of a pink layer, which in turn is underlain by a blue-green coloured basal layer (Bonilla et al. 2005). Microscopic observations show that the black layer is composed of large colonies with radially oriented filaments of *Tolypothrix* sp.; the pink layer is dominated by thin filamentous species (including *Leptolyngbya* and *Pseudanabaena)* and colonies of *Nostoc* spp., and the blue-green layer is composed of diverse filaments of both narrow and wide taxa from the order Oscillatoriales (*Lyngbya* spp.,

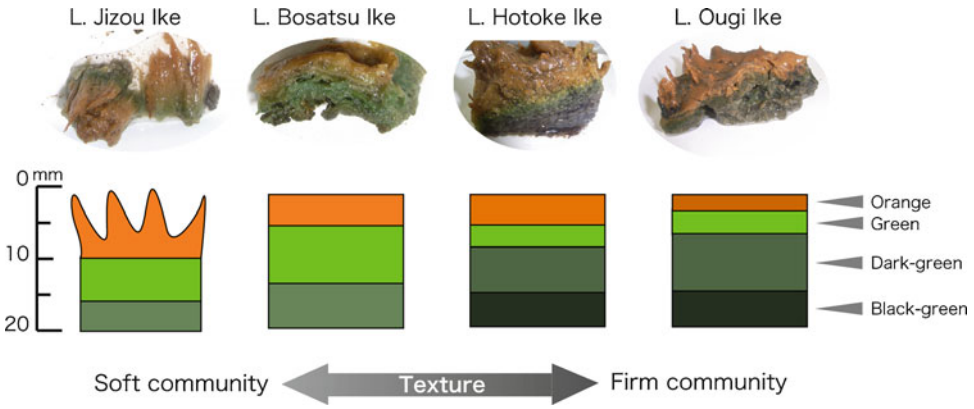

Fig. 13.7 Vertical sections through cyanobacterial mats from four lakes of the Skarvsnes area, East Antarctica. (From Tanabe et al. 2010; with permission)

Oscillatoria spp., *Leptolyngbya* sp.). Small diatoms such as *Achnanthes* and *Cymbella*, and the chlorophytes *Mougeotia* and *Closterium* are found in both the pink and blue-green layer. The absolute concentrations of scytonemin and its ratio to chl *a* are maximal at the top of the mat, and also the ratio of total carotenoid to chl a is maximal in the upper mat surface. The mats contain high concentrations of the cyanobacterial carotenoids canthaxanthin, echinenone, myxoxanthophyll, and a related glycoside closely resembling 4-keto-myxoxanthophyll, the most abundant carotenoid in the mats. The highest concentrations of violaxanthin and chl b have been found in the basal blue-green layer, indicating the increased importance of Chlorophyta in the lower community. Phycocyanin and allophycocyanin absorbance ratios to 750 nm are also highest in the basal stratum, indicative of the acclimation towards light-harvesting under dim light in this lower stratum (Bonilla et al. 2005).

The Skarvsnes area, east Antarctica, provides a compelling set of examples of pigmented microbial mats in the South Polar Region. This ice-free area contains about 50 shallow lakes and the pigment characteristics of mats in four of these lakes have been analysed in detail (Tanabe et al. 2010). As in the Arctic, the dominant pigments in the mats are from cyanobacteria (scytonemin, aphanizophyll, myxoxanthophyll, echinenone, canthaxanthin, zeaxanthin), followed by green algae (Chl b, violaxanthin, antheraxanthin, and lutein) and diatoms (Chl c, fucoxanthin and diadinoxanthin). There were large differences in colour zonation down the mat profile (Fig. 13.7). Scytonemin was found only in the top to middle sections, while Chl *a* concentrations were always highest in the deep, green layers. The total carotenoid, total xanthophyll (violaxanthin, diadinoxanthin, zeaxanthin, antheraxanthin, lutein) and scytonemin ratios to Chl *a* were highest in the uppermost, orange layer. In Larsemann Hills region (East Antarctica), 62 microbial mats were investigated regarding their pigment compositions. The variables affecting pigmentary composition. The authors identified lake depth, as a result of its influence on the light climate, as the most relevant variable defining the carotenoid and UV-protecting pigments composition in microbial mats (Hodgson et al. 2004).

13.4.3 PAR and UVR Responses

Polar cyanobacterial mats live under the two extremes of high and low irradiance conditions, and are capable of acclimating to both extremes. Under dim light, for example in the deep shade communities of McMurdo Dry Valley lakes, the high cellular concentrations of phycobiliproteins confer an ability to grow under extreme shade. Laboratory gas-exchange measurements by Hawes and Schwarz (1999, 2001) showed that the communities have an unusually efficient light-capturing capacity, with photosynthetic quantum yields close to the theoretical maximum. These results were subsequently confirmed by in situ oxygen micro-electrode measurements of photosynthesis in the mats under the perennial ice (Vopel and Hawes 2006).

At the other extreme, cyanobacterial mats exposed to full light in shallow or semi-aquatic ecosystems experience a range of light climate conditions. At the mat surface, cells are exposed to the full solar spectrum at high irradiance, but in the subsurface layers certain wavelengths are absorbed and irradiance is attenuated (Fig. 13.8). At only 2 mm from the mat surface the maximum irradiance reaching the cells may be only 5% of that at surface. This profile is caused by the high surface concentrations of photoprotective pigments which allow the cells deeper in the mat community to grow under milder conditions, free of UV radiation (e.g. Quesada et al. 1999; Tanabe et al. 2010). Experiments under controlled laboratory conditions showed that protective carotenoid pigmentation in an Antarctic mat-forming oscillatorian increased as a function of increasing UVR and PAR, and also with decreasing temperature (Roos and Vincent 1998). Similarly, a comparison of mat communities across northern

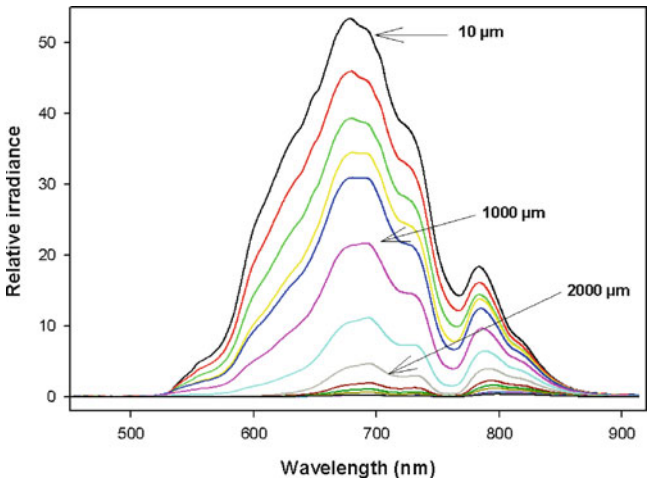

Fig. 13.8 Spectral irradiance in a depth profile within a cyanobacterial mat from Byers Peninsula (Livingston Island, Antarctica). Each line is an irradiance spectrum, at 200-µm depth intervals, relative to the surface irradiance. (A. Quesada, unpublished data)

Canada and Alaska showed that photoprotective pigments increased with increasing latitude, decreasing water temperature and increased UVR transparency of the overlying water (Bonilla et al. 2009). These cyanobacterial photoprotectants are ingested and bio-accumulated by some crustacean grazers on the mat communities, and may thereby provide some UV protection to higher trophic levels (Rautio et al. 2009).

As in microbial mats at lower latitudes, many oscillatorians in high latitude mats are capable of adjusting their position in the mats by their gliding motility (Vincent and Quesada 1994; Nadeau et al. 1999). Mat samples brought indoors sometimes visibly darken as the trichomes rich in light-capturing pigments, but deficient in light-protecting carotenoids and UV screening pigments, migrate to the surface. This migration behaviour allows the cells to avoid damaging exposure to UVR and bright PAR, while allowing them to rise to the surface and continue photosynthesis during periods of low incident irradiance, such as during freeze-up of the lake or pond habitat.

13.4.4 Nutrient Supply

Dissolved inorganic nutrients such as soluble reactive phosphorus and ammonium tend to be one or more orders of magnitude higher in the interstitial waters of polar mat communities than in the overlying water column (Vincent et al. 1993; Villeneuve et al. 2001), and there is also molecular evidence of that these mats are active sites of nutrient regeneration and scavenging (Varin et al. 2010). Metagenomic DNA analysis of 11.5 million base pairs showed that the ribosomal and protein-coding genes of two high Arctic ice shelf mat communities were dominated by Proteobacteria, not Cyanobacteria, implying a broad range of bacterial decomposition and nutrient recycling processes in addition to phototrophy. Viruses were also present (*Alpha-*, *Beta-*, *Gamma-proteobacteria* phages and cyanophages), and these also likely contribute to cellular lysis and recycling. The nitrogen-related genes were dominated by ammonium-assimilation systems, implying that the microbial mats are sites of intense mineralization. Nutrient scavenging systems including genes for transport proteins and enzymes converting larger molecules into more readily assimilated inorganic forms (allantoin degradation, cyanate hydrolysis, exophosphatases, phosphonatases). This analysis underscored the capability of polar microbial mat consortia to retain and recycle nutrients in an otherwise oligotrophic environment (Varin et al. 2010).

In some polar mat communities, nitrogen supply is supplemented by nitrogen fixation. For example, in a mat community for the McMurdo Ice Shelf, N_2-fixation was estimated to contribute about 30% of the total N budget (Fernández-Valiente et al. 2001). Molecular analysis of these communities has shown that although N_2-fixing bacteria other than cyanobacteria were represented in the DNA clone libraries for the nitrogenase gene nifH, gene expression was exclusively by the cyanobacterium *Nostoc* (Jungblut and Neilan 2010), in agreement to the physiological results by Fernández-Valiente et al. (2001) which indicated that N_2-fixation was light-dependent and most probably by heterocystous cyanobacteria. In other cyanobacterial mats from the same region, microscopic observations indicated that *Anabaena* sp. and *Nodularia* sp. were present and also likely contributed to N_2-fixation. Nitrogen-fixation by cyanobacterial communities is also well known from Arctic lakes and ponds (e.g. Bergmann and Welch 1990).

13.4.5 Salinity

Cyanobacteria mats in shallow waters are subject to pronounced variations in solute concentration throughout their growing season. During ice-formation, ions are excluded and a brine is formed in the water overlying the cyanobacterial mats, which are therefore exposed to major shifts in salinity (Schmidt et al. 1991; Hawes et al. 1999; Mueller and Vincent 2006). Many mat forming cyanobacteria appear to have a strong tolerance to these salinity variations (e.g. Lionard et al. 2012), however to an extent that varies among species, and conductivity is a variable that separates the distribution of taxa. For example, two morphotypes of *Oscillatoria priestleyi* appeared to be halophilic and restricted to high conductivity waters (4.2–55 mS cm^{-1}), while four *P. autumnale* morphotypes occurred over a wide range of conductivities (75–7,000 µS cm^{-1}) and one narrow-trichome oscillatorian morphotype was found only at the lowest conductivity, leading Broady and Kibblewhite (1991)

to suggest that different genotypes may have different salinity preferences.

Sabbe et al. (2004) conducted a similar analysis at Larsemann Hills and Bølingen Islands (East Antarctica) and found a relationship between diatom composition but not cyanobacterial morphospecies and salinity. However, in a subsequent more detailed study based on molecular methods (DGGE analysis) at five ice-free oases, salinity emerged as a key variable in the ordination analyses for separating different cyanobacterial genotypes (Verleyen et al. 2010). Similarly, in a clone library analysis of cyanobacterial distribution on the McMurdo Ice Shelf, salinity was an important discriminating variable (Jungblut et al. 2005). When a large pond drained on this ice shelf leaving 13 residual ponds, there was little difference in biomass among the ponds two seasons later; however there were differences in community structure that appeared to be related to differences in salinity (Sutherland 2009).

13.4.6 Freeze-Up and Desiccation Tolerance

Cyanobacterial mats in the polar regions may experience complete freeze-up of their aquatic environment, or evaporation to dryness given the low precipitation-evaporation balance at many sites. Both of these conditions imply an increase in osmolarity, through freeze- or evaporative- concentration. For example, in a study on Ross Island ponds, the benthic communities experienced relatively freshwater conditions in late summer but salinities up to five times that of seawater, and liquid water temperatures down to −12°C, during the final stages of freeze-up in winter (Schmidt et al. 1991). Ice crystal formation that accompanies freeze-up can also mechanically damage cell membranes, particularly if the crystals are formed within the cells (Vincent 1988). Mat-forming cyanobacteria in the polar regions have a variety of strategies to minimize these osmotic and mechanical stresses including production of mucopolysaccharides (exopolymeric substances) and compatible solutes (Vincent 2007, and refs therein). Experiments on Antarctic microbial mats have shown a high level of tolerance to desiccation, but to an extent that differs among different mat types (Hawes et al. 1992). The extreme tolerance of cyanobacteria as a group to osmotic stress and desiccation is also suggested by their ability to tolerate very low water potentials (Wynn-Williams 2000). Recovery from desiccation conditions appears to vary among species and polar communities. In Antarctica, some *Nostoc* mats were began respiration and photosynthesis only 10 min after rewetting, while *Phormidium* based microbial mats did not achieve complete recovery even after 10 days of rewetting (Hawes et al. 1992). Metagenomic studies have revealed a broad spectrum of stress genes in both Arctic and Antarctic cyanobacterial mats, including sigma B genes that may be involved in acclimating to freeze-up and osmotic stresses (Varin et al. 2012).

13.4.7 Controlling Factors

Cyanobacteria isolated from mats in the polar regions have been shown to tolerate a wide range of conditions, and to maintain slow net growth despite the frigid ambient temperatures (Tang and Vincent 1999, 2000). The large standing stocks of benthic cyanobacteria in many polar freshwater environments imply that conditions are highly favourable for their net accumulation, in marked contrast to the typically low biomass of planktonic phototrophs in the overlying water column. However, there are large variations between sites, with the benthic communities ranging from thin sub-millimetre biofilms that require a microscope to detect, to microbial mats that are several cm in thickness. What factors contribute to such a contrast between the plankton and the benthos, and to the pronounced site-to-site variability?

Standing stocks of biomass at any point in time are the result of the integrated balance of production and loss (P/L) processes at timescales ranging from days to decades. Primary production rates in cyanobacterial mats from both polar regions mostly fall within a narrow range (1–10 μg $C\,cm^{-2}\,h^{-1}$; Quesada et al. 2008 and references therein). Therefore, the markedly different extents of mat accumulation at different locations within the polar regions may be explained by differences in the P/L balance at each site, including the growing period that is determined by seasonal irradiance and the duration of liquid water supply. The latter is somewhat longer than expected based on freshwater, since ice formation results in increased salinities (see above) that extend the liquid conditions to subzero temperatures. In McMurdo Ice Shelf ponds at latitude 78°S, liquid water was detected at the bottom of <1 m deep ponds as late in the season as April (Hawes et al. 1999; Hawes et al. 2011). However, at that time of year and latitude, irradiance is extremely low and the physiological maintenance costs for phototrophs may be larger than their cellular gains by photosynthesis. Differences in the extent of overwintering biomass may also contribute the variability in standing stocks of microbial mats.

The availability of liquid water is the first prerequisite for microbial activity, and in many polar aquatic environments this resource is highly seasonal; for example, in stream beds that dry up and lakes that freeze to the bottom. Mat-forming cyanobacteria in these habitats seem highly tolerant of these conditions of freeze-up and desiccation (see above), and are able to maintain large overwintering biomass stocks that provide them with a competitive advantage after melt-out the next season. Light availability for photosynthesis is highly seasonal throughout the polar regions, and the underwater solar radiation regime during the growing season varies from prolonged dim light conditions under snow and ice to 24 h of sunshine, including high UV exposure. As noted above, cyanobacteria have a variety of pigment strategies that allows

them to minimize photodamage (even when desiccated) and maximize photon capture. Low temperature is another Blackman-type limitation on production rates, given that most polar cyanobacteria have high temperature optima and show extremely slow growth at ambient temperatures (Tang et al. 1997; Velázquez et al. 2011).

In the benthic environment of polar lakes and rivers, several features result in reduced loss rates and thereby push these communities towards slow net accumulation. Grazing losses to benthic herbivores are minimal in many of these waters as a result of the short growing season for invertebrates to complete their life cycles, and other inhospitable features of the polar environment that prevent colonisation by most animal species. Some crustacean species do occur in Antarctic and High Arctic ponds, including fairy shrimps such as *Branchinecta* and *Artemiopsis* that feed at least partially on cyanobacterial mats (Rautio and Vincent 2006). In many lakes and ponds in the Arctic, including the northernmost lake in Canada (Bonilla et al. 2005), chironomid larvae also feed on the mats, and within the mats several species of microinvertebrates are typically found including rotifers, nematodes, tardigrades and flatworms. Some cyanobacteriovorous ciliates are also frequent within cyanobacterial mats (Petz et al. 2007), mostly feeding on thin oscillatorians such as *Leptolyngbya*. These protozoa and invertebrate communities, however, are limited in biomass and duration of feeding activity, with presumed little impact on the standing crop of microbial biomass, although this has yet to be fully tested. The cold temperatures also inhibit bacterial degradation processes, and analyses of bacterial growth rates and respiration generally indicate slow rates of mineralisation of the organic biomass to CO_2. This in combination with adequate nutrient supply may be the reason for the development of the thickest mats in extreme cold environments, for example in ice shelf meltponds and in the phytobenthos of frigid Antarctic lakes. Viruses are known to occur within the mats (Vincent et al. 2000) and include cyanophage (Varin et al. 2010), however loss rates by viral lysis of cyanobacterial cells have not been quantified to date.

Limited nutrient supply can result in both Liebig (yield) and Blackman (growth rates) limitation effects, and in this regard there is a striking contrast between the plankton and phytobenthos of polar lakes. High latitude lake phytoplankton are typically subject to severe nutrient limitation, and respond strongly to nitrogen and or phosphorus addition. In contrast, microbial mats are zones of nutrient scavenging and regeneration, with microenvironmental nutrient concentrations that are much higher than the bulk concentrations in their overlying environments (see above). A series of bioassays at Ward Hunt Lake in the Canadian High Arctic provided evidence of these contrasting nutrient regimes: benthic cyanobacterial mats showed no significant photosynthetic or biomass response to nutrient enrichment over 4–8 days, while the phytoplankton increased several fold after nutrient addition (Bonilla et al. 2005). It is possible that nutrient enrichment at longer timescales (months to years) may favour the gradual accretion of biomass in perennial microbial mats.

Field observations suggest that physical attrition processes may be more effective in controlling cyanobacterial mat biomass than biological loss factors. For example, the incorporation of gas bubbles within the mat structure increases the buoyancy of large portions of mats (Wharton et al. 1983). These lift-off mats float up from the sediments and are then pushed to the lake-edge by the wind, and large accumulations of dead microbial mats are sometimes seen washed up on the shore. Another physical effect is scouring by the lake ice during melt-out, which might be responsible of the absence of microbial mats from the inshore waters of many polar lakes. Fast flowing or sediment laden streams will also lose their microbial mats by scouring, while ponds and streams that dry up in late summer, or ablate to dryness over winter, may lose microbial mat biomass by wind erosion.

13.5 Conclusions

Cyanobacteria are poorly represented in polar marine environments, but they are enormously successful in high latitude lakes, ponds, wetlands and rivers where they may be the biomass and production dominants. These organisms tolerate the broad range of extreme conditions experienced in the Arctic and Antarctica, including freeze-thaw cycles, variable osmolarity, persistent low temperatures and extremes of irradiance including UV radiation. They are especially successful in the benthic environment where they produce nutrient-rich mat communities that contain taxonomically and functionally diverse populations of micro-organisms. Each of the three ecological groups is highly responsive to temperature, and cyanobacteria are likely to prosper from ongoing climate change in the polar regions.

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References

Andersen DT, Sumner DY, Hawes I, Webster-Brown J, McKay CP (2011) Discovery of large conical stromatolites in Lake Untersee, Antarctica. Geobiology 9:280–293

Asthana RK, Deepali A, Tripathi MK, Srivastava A, Singh AP, Singh SP, Nath G, Srivastava R, Srivastava BS (2009) Isolation and identification of a new antibacterial entity from the Antarctic cyanobacterium *Nostoc* CCC 537. J Appl Phycol 21:81–88

Bergeron M, Vincent WF (1997) Microbial food web responses to phosphorus and solar UV radiation in a subarctic lake. Aquat Microb Ecol 12:239–249

Bergmann MA, Welch HE (1990) Nitrogen-fixation by epilithic periphyton in small arctic lakes in response to experimental nitrogen and phosphorus fertilization. Can J Fish Aquat Sci 47:1545–1550

Bonaventura SM, Vinocur A, Allende L, Pizarro H (2006) Algal structure of the littoral epilithon in lentic water bodies at Hope Bay, Antarctic Peninsula. Polar Biol 29:668–680

Bonilla S, Villeneuve V, Vincent WF (2005) Benthic and planktonic algal communities in a high arctic lake: pigment structure and contrasting responses to nutrient enrichment. J Phycol 41:1120–1130

Bonilla S, Rautio M, Vincent WF (2009) Phytoplankton and phytobenthos pigment strategies: implications for algal survival in the changing Arctic. Polar Biol 28:846–861

Bottos EM, Vincent WF, Greer CW, Whyte LG (2008) Prokaryotic diversity of arctic ice shelf microbial mats. Environ Microbiol 10:950–966. doi:10.1111/j.1462-2920.2007.01516.x

Brambilla E, Hippe H, Hagelstein A, Tindall BJ, Stackebrandt E (2001) 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles 5:23–33

Broady PA, Kibblewhite A (1991) Morphological characterization of Oscillatoria (Cyanobacteria) from Ross Island and southern Victoria Land, Antarctica. Antarct Sci 3:35–45

Comte K, Sabacka M, Carre-Miouka A, Elster J, Komárek J (2007) Relationships between the Arctic and the Antarctic cyanobacteria; three *Phormidium*-like strains evaluated by a polyphasic approach. FEMS Microbiol Ecol 59:366–376

Cottrell MT, Kirchman DL (2009) Photoheterotrophic microbes in the Arctic Ocean in summer and winter. Appl Environ Microbiol 75:4958–4966

Christner BC, Kvitko BH II, Reeve JN (2003) Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole. Extremophiles 7:177–183

de los Ríos A, Ascaso C, Wierzchos J, Fernández-Valiente E, Quesada A (2004) Microstructural characterization of cyanobacterial mats from the McMurdo Ice Shelf, Antarctica. Appl Environ Microbiol 70:569–580

Doolittle DF, Li WKW, Wood AM (2008) Wintertime abundance of picoplankton in the Atlantic sector of the Southern Ocean. Nova Hedwig 133:147–160

Dorador C, Vila I, Imhoff JF, Witzel KP (2008) Cyanobacterial diversity in Salar de Huasco, a high altitude saline wetland in northern Chile: an example of geographical dispersion? FEMS Microbiol Ecol 64:419–432

Fernández-Carazo R, Hodgson DA, Convey P, Wilmotte A (2011) Low cyanobacterial diversity in biotopes of the Transantarctic Mountains and Shackleton Range (80–82ºS), Antarctica. FEMS Microbiol Ecol 77:503–517

Fernández-Valiente E, Quesada A, Howard-Williams C, Hawes I (2001) N_2-fixation in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Microb Ecol 42:338–349

Fernández-Valiente E, Camacho A, Rochera C, Rico E, Vincent WF, Quesada A (2007) Community structure and physiological characterization of microbial mats in Byers Peninsula, Livingston Island (South Shetland Islands, Antarctica). FEMS Microbiol Ecol 59:377–385

Foreman CM, Sattler B, Mikucki JA, Porazinska DL, Priscu JC (2007) Metabolic activity and diversity of cryoconites in the Taylor Valley, Antarctica. J Geophys Res Biogeosci 112(G4):1–11, G04S32

Gerdel RW, Drouet F (1960) The cyanobacteria of the Thule area, Greenland. Trans Am Microsc Soc 79:256–272

Gradinger R, Lenz J (1995) Seasonal occurrence of picocyanobacteria in the Greenland Sea and central Arctic Ocean. Polar Biol 15:447–452

Hawes I (1990) Eutrophication and vegetation development in maritime Antarctic lakes. In: Kerry KR, Hempel G (eds) Antarctic ecosystems: ecological change and conservation. Springer, Berlin, pp 83–90, 427 pp

Hawes I, Schwarz AM (1999) Photosynthesis in an extreme shade environment: benthic microbial mats from Lake Hoare, a permanently ice-covered Antarctic lake. J Phycol 35:448–459

Hawes I, Schwarz AM (2001) Absorption and utilization of irradiance by cyanobacterial mats in two ice-covered Antarctic lakes with contrasting light climates. J Phycol 37:5–15

Hawes I, Howard-Williams C, Vincent WF (1992) Desiccation and recovery of Antarctic cyanobacterial mats. Polar Biol 12:587–594

Hawes I, Safi K, Sorrell B, Webster-Brown J, Arscott D (2011). Summer-winter transitions in Antarctic ponds I. The physical environment. Antarctic Sci 23:235–242

Hawes I, Smith R, Howard-Williams C, Schwarz AM (1999) Environmental conditions during freezing, and response of microbial mats in ponds of the McMurdo Ice Shelf, Antarctica. Antarct Sci 11:198–208

Heath MW, Wood SA, Ryan KG (2010) Polyphasic assessment of fresh-water benthic mat-forming cyanobacteria isolated from New Zealand. FEMS Microbiol Ecol 73:95–109

Hitzfeld BC, Lampert CS, Spaeth N, Mountfort D, Kaspar H, Dietrich DR (2000) Toxin production in cyanobacterial mats from ponds on the McMurdo Ice shelf, Antarctica. Toxicon 38:1731–1748

Hodgson DA, Vyverman W, Verleyen E, Sabbe K, Leavitt PR, Taton A, Squier AH, Keely BJ (2004) Environmental factors influencing the pigment composition of in situ benthic microbial communities in east Antarctic lakes. Aquat Microb Ecol 37:247–263

Imura S, Bando T, Saito S, Seto K, Kanda H (1999) Benthic moss pillars in Antarctic lakes. Polar Biol 22:137–140

Jungblut AD, Neilan BA (2010) NifH gene diversity and expression in a microbial mat community on the McMurdo Ice Shelf, Antarctica. Antarct Sci 22:117–122

Jungblut AD, Hawes I, Mountfort D, Hitzfeld B, Dietrich DR, Burns BP, Neilan BA (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environ Microbiol 7:519–529

Jungblut AD, Lovejoy C, Vincent WF (2010) Global distribution of cyanobacterial ecotypes in the cold biosphere. ISME J 4:191–202

Kalff J, Kling J, Holmgren SH, Welch HE (1975) Phytoplankton, phytoplankton growth and biomass cycles in an unpolluted and in a polluted lake. Verh Int Ver Limnol 19:487–495

Komárek J, Elster J (2008) Ecological background of cyanobacterial assemblages of the northern part of James Ross Island, Antarctica. Pol Polar Res 29:17–32

Komárek J, Elster J, Komárek O (2008) Diversity of the cyanobacterial microflora of the northern part of James Ross Island, NW Weddell Sea, Antarctica. Polar Biol 31:853–865

Laurion I, Vincent WF (1998) Cell size versus taxonomic composition as determinants of UV sensitivity in natural phytoplankton communities. Limnol Oceanogr 43:1774–1779

Leslie A (1879) The Arctic voyages of Adolf Erik Nordenskiöld. MacMillan and Co., London

Li WKW (2009) From cytometry to macroecology: a quarter century quest in microbial oceanography. Aquat Microb Ecol 57:239–251

Lionard M, Péquin B, Lovejoy C, Vincent WF (2012) Benthic cyanobacterial mats in the High Arctic: Multi-layer structure and fluorescence responses to osmotic stress. Frontiers Aquat Microbiol. doi:10.3389/fmicb.2012.00140

Lovejoy C, Vincent WF, Bonilla S, Roy S, Martineau MJ, Terrado R, Potvin M, Massana R, Pedros-Alio C (2007) Distribution, phylogeny and growth of cold-adapted picoprasinophytes in arctic seas. J Phycol 43:78–89

Mehnert G, Leunert F, Cirés S, Jöhnk KD, Rücker J, Nixdorf B, Wiedner C (2010) Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. J Plankton Res 32:1009–1021

Michaud AB, Sabacka M, Priscu JC (2012) Cyanobacterial diversity across landscape units in a polar desert: Taylor Valley, Antarctica. FEMS Microbiol Ecol. doi:10.1111/j.1574-6941.2012.01297.x

Mueller DR, Vincent WF (2006) Microbial habitat dynamics and ablation control on the Ward Hunt Ice Shelf. Hydrol Process 20:857–876

Mueller DR, Vincent WF, Bonilla S, Laurion I (2005) Extremophiles, extremotrophs and broadband pigmentation strategies in a high arctic ice shelf ecosystem. FEMS Microbiol Ecol 53:73–87

Murray J (1910) On collecting at Cape Royds. In: Murray J (ed) British Antarctic expedition 1907–1909 reports on scientific expeditions, vol 1, Biology. Heinemann, London, pp 1–15

Nadeau T, Howard-Williams C, Castenholz RW (1999) Effects of solar UV and visible irradiance on photosynthesis and vertical migration of *Oscillatoria* sp. (cyanobacteria) in an Antarctic microbial mat. Aquat Microb Ecol 20:231–243

Nakai R, Abe T, Baba T, Imura S, Kagoshima H, Kanda H, Kanekiyo A, Kohara Y, Koi A, Nakamura K, Narita T, Niki H, Yanagihara K, Naganuma T (2012) Microflorae of aquatic moss pillars in a freshwater lake, East Antarctica, based on fatty acid and 16S rRNA gene analyses. Polar Biol 35:425–433

Novis PM, Smissen RD (2006) Two generic and ecological groups of *Nostoc commune* in Victoria Land, Antarctica, revealed by AFLP analysis. Antarct Sci 18:573–581

Parker BC, Wharton RA (1985) Physiological ecology of blue-green algal mats (modern stromatolites) in Antarctic oasis lakes. Arch Hydrobiol Suppl 71:331–348

Petz W, Valbonesi A, Schiftner U, Quesada A, Ellis-Evans JC (2007) Ciliate biogeography in Antarctic and Arctic freshwater ecosystems: endemism or global distribution of species? FEMS Microbiol Ecol 59:396–408

Powell LM, Bowman JP, Skerratt JH, Franzmann PD, Burton HR (2005) Ecology of a novel *Synechococcus* clade occurring in dense populations in saline Antarctic lakes. Mar Ecol Prog Ser 291:65–80

Priscu JC, Fritsen CH, Adams EE, Giovannoni SJ, Paerl HW, McKay CP, Doran PT, Gordon DA, Lanoil BD, Pinckney JL (1998) Perennial Antarctic lake ice: an oasis for life in a polar desert. Science 280:2095–2098

Proteau PJ, Gerwick WH, García-Pichel F, Castenholz R (1993) The structure of scytonemin: an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. Experientia 49:825–829

Quayle WC, Peck LS, Peat H, Ellis-Evans JC, Harrigan PR (2002) Extreme responses to climate change in Antarctic lakes. Science 295:645

Quesada A, Vincent WF (1997) Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. Eur J Phycol 32:335–342

Quesada A, Vincent WF (2012) Cyanobacteria in the cryosphere: snow, ice and extreme cold. In: Whitton BA (ed) Ecology of cyanobacteria II. Springer, Dordrecht, pp, pp

Quesada A, Vincent WF, Lean DRS (1999) Community and pigment structure of Arctic cyanobacterial assemblages: the occurrence and distribution of UV-absorbing compounds. FEMS Microbiol Ecol 28:315–323

Quesada A, Fernández-Valiente E, Hawes I, Howard-Williams C (2008) Benthic primary production in polar lakes and rivers. In: Vincent WF, Laybourn-Parry J (eds) Polar lakes and rivers. Oxford University Press, Oxford, pp 179–196, 327 pp

Rae R, Vincent WF (1998) Phytoplankton production in subarctic lake and river ecosystems: development of a photosynthesis-temperature-light model. J Plankton Res 20:1293–1312

Rautio M, Vincent WF (2006) Benthic and pelagic food resources for zooplankton in shallow high-latitude lakes and ponds. Freshw Biol 51:1038–1052

Rautio M, Bonilla S, Vincent WF (2009) UV photoprotectants in arctic zooplankton. Aquat Biol 7:93–105

Rezanka T, Nedbalova L, Elster J, Cajthaml T, Sigler K (2009) Very-long-chain iso and anteiso branched fatty acids in N-acylphosphatidylethanolamines from a natural cyanobacterial mat of *Calothrix* sp. Phytochemistry 70:655–663

Roos J, Vincent WF (1998) Temperature dependence of UV radiation effects on Antarctic cyanobacteria. J Phycol 34:78–85

Sabbe K, Hodgson DA, Verleyen E, Taton A, Wilmotte A, Vanhoutte K, Vyverman W (2004) Salinity, depth and the structure and composition of microbial mats in continental Antarctic lakes. Freshw Biol 49:296–319

Schiaffino MR, Unrein F, Gasol J, Farias ME, Estevez C, Balague V, Izaguirre I (2009) Comparative analysis of bacterioplankton assemblages from maritime Antarctic freshwater lakes with contrasting trophic status. Polar Biol 32:923–936

Schindler DW (1974) Eutrophication in the high Arctic – Meretta Lake, Cornwallis Island (75°N Lat.). J Fish Res Board Can 31:647–662

Schmidt S, Moskal W, Demora SJ, Howard-Williams C, Vincent WF (1991) Limnological properties of Antarctic ponds during winter freezing. Antarct Sci 3:379–388

Shukla SP, Kashyap AK (2003) An assessment of biopotential of three cyanobacterial isolates from Antarctic for carotenoid production. Indian J Biochem Biophys 40:362–366

Shukla SP, Singh JS, Kashyap S, Giria DD, Kashyap AK (2008) Antarctic cyanobacteria as a source of phycocyanin. An assessment. Indian J Mar Sci 37:446–449

Singh SM, Elster J (2007) Cyanobacteria in Antarctic lake environments: a mini-review. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 303–320, 811 pp

Singh SP, Kumari S, Rastogi RP, Singh KL, Richa SRP (2010) Photoprotective and biotechnological potentials of cyanobacterial sheath pigment, scytonemin. Afr J Biotechnol 9:580–588

Spaulding SA, McKnight DMM, Smith RL, Dufford R (1994) Phytoplankton population dynamics in perennially ice-covered Lake Fryxell, Antarctica. J Plankton Res 16:527–541

Strunecký O, Elster J, Komárek J (2010) Phylogenetic relationships between geographically separate *Phormidium* cyanobacteria: is there a link between north and south polar regions? Polar Biol. doi:10.1007/s00300-010-0834-8

Sutherland S (2009) Microbial mat communities in response to recent changes in the physiochemical environment of the meltwater ponds on the McMurdo Ice Shelf, Antarctica. Polar Biol 32:1023–1032

Tanabe Y, Ohtan S, Kasamatsu N, Fukuchi M, Kudoh S (2010) Photophysiological responses of phytobenthic communities to the strong light and UV in Antarctic shallow lakes. Polar Biol 33:85–100

Tang EPY, Vincent WF (1999) Strategies of thermal adaptation by high latitude cyanobacteria. New Phytol 142:315–323

Tang EPY, Vincent WF (2000) Effects of daylength and temperature on the growth and photosynthesis of an Arctic cyanobacterium *Schizothrix calcicola* (Oscillatoriaceae). Eur J Phycol 35:263–272

Tang EPY, Tremblay R, Vincent WF (1997) Cyanobacterial dominance of polar freshwater ecosystems: are high latitude mat-formers adapted to the low temperature environment? J Phycol 33:171–181

Taton A, Grubisic S, Brambilla E, De Wit R, Wilmotte A (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl Environ Microbiol 69:5157–5169

Taton A, Grubisic S, Balthazart P, Hodgson DA, Laybourn-Parry J, Wilmotte A (2006a) Biogeographical distribution and ecological range of benthic cyanobacteria in East Antarctic lakes. FEMS Microbiol Ecol 57:272–289

Taton A, Grubisic S, Ertz D, Hodgson DA, Piccardi R, Biondi N, Tredici MR, Mainini M, Losi D, Marinelli F, Wilmotte A (2006b) Polyphasic study of Antarctic cyanobacterial strains. J Phycol 42:1257–1270

Taylor G (1916) With Scott. The silver lining. Dodd Mead and Co., New York

Toro M, Camacho A, Rochera C, Rico E, Bañón M, Fernández-Valiente E, Marco E, Justel A, Avendaño MC, Ariosa Y, Vincent WF, Quesada A (2007) Limnological characteristics of the freshwater ecosystems of Byers Peninsula, Livingston Island, in maritime Antarctica. Polar Biol 30:635–649

Vallières C, Retamal L, Osburn C, Vincent WF (2008) Bacterial production and microbial food web structure in a large arctic river and the coastal Arctic Ocean. J Mar Syst 74:756–773

Van Hove P, Vincent WF, Galand PE, Wilmotte A (2008) Abundance and diversity of picocyanobacteria in high arctic lakes and fjords. Algol Stud 126:209–227

Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J (2010) Metagenomic profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems. Limnol Oceanogr 55:1901–1911

Varin T, Lovejoy C, Jungblut AD, Vincent WF, Corbeil J (2012) Metagenomic analysis of stress genes in microbial mat communities from extreme Arctic and Antarctic environments. Appl Environ Microbiol 78:49–559

Veillette J, Martineau M-J, Antoniades D, Vincent WF (2011) Effects of loss of perennial lake ice on mixing and phytoplankton dynamics: insights from High Arctic Canada. Ann Glaciol 51:56–70

Velázquez D, Rochera C, Camacho A, Quesada A (2011) Temperature effects on Antarctic non-marine phototrophic communities. Polar Biol 34:1045–1055

Verleyen E, Sabbe K, Hodgson DA, Grubisic S, Taton A, Cousin S, Wilmotte A, De Wever A, Van der Gucht K, Vyverman W (2010) Structuring effects of climate-related environmental factors on Antarctic microbial mat communities. Aquat Microb Ecol 59:11–24

Vézina S, Vincent WF (1997) Arctic cyanobacteria and limnological properties of their environment: Bylot Island, Northwest Territories, Canada. (73°N, 80°W). Polar Biol 17:523–534

Villeneuve V, Vincent WF, Komárek J (2001) Community structure and microhabitat characteristics of cyanobacterial mats in an extreme high Arctic environment: Ward Hunt Lake. In: Elster J, Seckbach J, Vincent WF, Lhotsky O (eds) Algae and extreme environments. Nova Hedwig Beih 123:199–224

Vincent WF (1988) Microbial ecosystems of Antarctica. Cambridge University Press, Cambridge

Vincent WF (2000) Cyanobacterial dominance in the polar regions. In: Whitton BA, Potts M (eds) Ecology of the cyanobacteria: their diversity in time and space. Kluwer, Dordrecht, pp 321–340, 669 pp

Vincent WF (2007) Cold tolerance in cyanobacteria and life in the cryosphere. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Heidelberg, pp 289–304, 811 pp

Vincent WF (2009) Cyanobacteria. In: Likens GE (ed) Encyclopedia of inland waters, vol 3. Elsevier, Oxford, pp 226–232, 859 pp

Vincent WF (2010) Microbial ecosystem responses to global warming in the Arctic. ISME J 4:191–202. doi:10.1038/ismej.2010.108

Vincent WF, Downes MT, Castenholz RW, Howard-Williams C (1993) Community structure and pigment organisation of cyanobacteria-dominated microbial mats in Antarctica. Eur J Phycol 28:213–221

Vincent WF, Gibson JAE, Pienitz R, Villeneuve V, Broady PA, Hamilton PB, Howard-Williams C (2000) Ice shelf microbial ecosystems in the high Arctic and implications for life on snowball Earth. Naturwissenschaften 87:137–141

Vincent WF, Mueller DR, Bonilla S (2004a) Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the High Arctic. Cryobiology 48:103–112

Vincent WF, Mueller D, Van Hove P, Howard-Williams C (2004b) Glacial periods on early Earth and implications for the evolution of life. In: Seckbach J (ed) Origins: genesis, evolution and diversity of life. Kluwer Academic Publishers, Dordrecht, pp 481–499, 707 pp

Vincent WF, Quesada A (1994) Ultraviolet radiation effects on cyanobacteria: implications for Antarctic microbial ecosystems. In: Weiler S, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. American Geophysical Union, Washington, DC, pp 111–124, 257 pp

Vincent WF, Vincent CL (1982) Factors controlling algal production in Lake Vanda (77°S). Can J Fish Aquat Sci 39:1602–1609

Vopel K, Hawes I (2006) Photosynthetic performance of benthic microbial mats in Lake Hoare, Antarctica. Limnol Oceanogr 51: 1801–1812

Waleron M, Waleron K, Vincent WF, Wilmotte A (2007) Allochthonous inputs of riverine picocyanobacteria to coastal waters in the Arctic Ocean. FEMS Microbiol Ecol 59:356–365

Welch HE, Legault JA, Kling HJ (1989) Phytoplankton, nutrients and primary production in fertilized and natural lakes at Saqvaqjuaq, NWT. Can J Fish Aquat Sci 46:90–107

Wharton RA Jr, Parker BC, Simmons GM Jr (1983) Distribution, species composition and morphology of algal mats in Antarctic dry valley lakes. Phycologia 22:355–365

Wood SA, Mountfort D, Selwood AI, Holland PT, Puddick J, Cary SC (2008) Widespread distribution and identification of eight novel microcystins in Antarctic cyanobacterial mats. Appl Environ Microbiol 74:7243–7251

Wynn-Williams DD (2000) Cyanobacteria in deserts- life at the limit? In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 341–366, 669 pp

Cyanobacteria in the Cryosphere: Snow, Ice and Extreme Cold

14

Antonio Quesada and Warwick F. Vincent

Contents

Summary

This chapter explores the occurrence and dominance of cyanobacteria in some of the harshest environments on earth, the cryosphere, where extreme cold and the near absence of liquid water provide severe constraints on growth and survival. They are present in ice-based ecosystems including snow, glacier ice, lake ice and ice-shelves, and sometimes achieve remarkably high biomass concentrations. Cold desert ecosystems in the Arctic and Antarctica also contain a variety of habitats colonized by cyanobacteria, although their diversity is low, and similar taxa are present in different geographic locations under similar ecological conditions. The strategy for microbial success in these environments is not adaptation towards optimal growth at low temperatures, but instead rests on tolerance to environmental extremes. An ability to survive prolonged dormancy is also an important feature accounting for the widespread occurrence of cyanobacteria in these environments.

14.1 Introduction

Cyanobacteria are near-ubiquitous organisms that occur in most sun-exposed ecosystems on Earth. In many ecosystems cyanobacteria are seasonally dominant, for example eutrophic lakes in summers in temperate regions. In extreme ecosystems such as high salinity (Chap. 15), thermal (Chap. 3) and oil polluted environments (Chap. 16), cyanobacteria can become exceptionally abundant and dominate through much or all of the year. Among these extreme environments is the cryosphere, which contains some of the harshest conditions for life as a result of extreme cold and the scarcity of liquid water. The cryosphere is defined here as the regions where temperatures remain below 0°C during most of the year, notably alpine regions and both Polar Regions, the Arctic and Antarctica. Most potential habitats in the cryosphere are within or covered by ice. However, some locations are snow and ice-free for much of the year – alpine and polar deserts.

A. Quesada (✉)
Departamento de Biologia, Facultad de Ciencias,
Universidad Autonoma de Madrid, 28049 Madrid, Spain
e-mail: antonio.quesada@uam.es

W.F. Vincent
Departement de Biologie, Université de Laval,
Quebec City, QC, G1V OA6, Canada
e-mail: warwick.vincent@bio.ulaval.ca

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These latter environments have been considered analogues for the possible development of life on frozen planets or moons (Wynn-Williams 2000), while the ice-bound ecosystems have provided insights into how life may have survived during global freeze-up events on early Earth (Vincent and Howard-Williams 2000). Cyanobacteria are frequently found in the ice as well as cold desert habitats, and use a variety of strategies to mitigate the harshness of their surroundings (Vincent 2007). For example, some communities live inside rocks where the humidity can be higher and the thermal variation is buffered (Nienow and Friedmann 1993), while others form dark-colored mats on or within ice that absorbs sunlight, which increases the temperature enough to melt some ice in summer and provide liquid water conditions (Mueller and Pollard 2004). Cyanobacteria inhabiting the cryosphere do not appear to be very diverse, and are mostly comprised of a few morphospecies (Nienow and Friedmann 1993; Wynn-Williams 2000). Global molecular studies are now well underway, and are providing some evidence of locally restricted (endemic) taxa (Taton et al. 2003), but also the cosmopolitan distribution of closely related genotypes throughout the cryosphere (Jungblut et al. 2010).

Contrary to expectation, cyanobacteria growing in the cryosphere are not cold-adapted psychrophiles (Vincent 2000). Instead, and with very few exceptions (Nadeau and Castenholz 2000), they are psychrotrophic (cold-tolerant) organisms, able to survive and slowly grow at low temperatures, but with temperature optima well above the temperatures found at their habitats (Tang et al. 1997). This lack of adaptative tuning to a low temperature regime may be an optimal strategy in these ecosystems where temperatures can fluctuate widely in few hours (Fig. 14.1), reaching high values at which organisms fully adapted for growth in the cold could suffer severe physiological stress and mortality. Under these suboptimal conditions cyanobacterial growth rate is modest (Vincent 2007), but in spite of this, they can achieve conspicuously large standing crops, and they colonize most habitats (see also Chap. 13).

Apart from extreme cold, cyanobacteria growing in the cryosphere also need to cope with the consequences of freeze-up and ice formation. During the latter, solutes are excluded from the growing ice, resulting in osmotic stress on cells. If the ice crystals are formed inside the cells, they can lead to the physical disruption of the membranes, and destruction of cell integrity (Vincent 2007). Cyanobacteria from the Polar Regions have a variety of strategies to reduce the damage produced by both osmotic shock and physical disruption. Exopolysaccharides are thought to be a primary mechanism of protection by reducing the water loss and by restricting ice crystal formation to sites outside the cells (Wynn-Williams 2000; Vincent 2007). Some cyanobacteria also produce osmoregulatory intracellular proteins, which regulate the osmotic stress imposed by desiccation and antifreeze compounds which are thought to act as cryoprotectants (Raymond and Fritsen 2000). Some of the information in this chapter is based on the extensive review by Wynn-Williams (2000). Some of the habitats and communities described below are illustrated in the online article associated with this book chapter (Quesada and Vincent: Cyanobacterial diversity and dominance in the cryosphere).

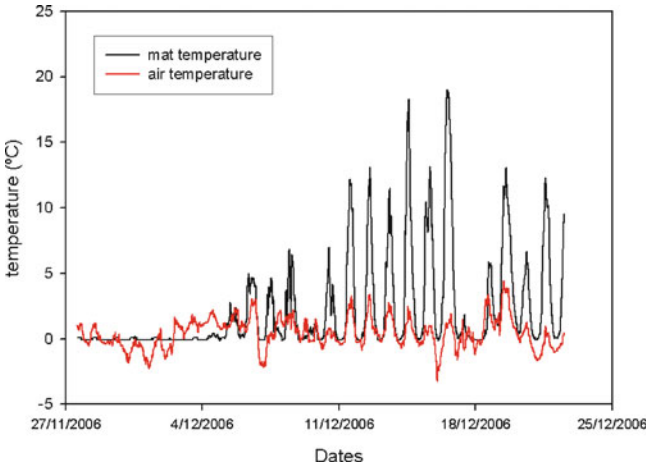

Fig. 14.1 Daily temperature variations in November–December 2006 in a microbial mat in Byers Peninsula (Livingston Island, South Shetland Islands) (Unpublished results, D. Velázquez, personal communication, 2007)

14.2 Polar Desert Ecosystems

Cold deserts occur in both the North and South Polar Regions, and contain some of the most inhospitable environments for life. Besides suffering the rigours of extreme cold, the persistent lack of liquid water and repeated freeze-thaw cycles allow only the most resistant organisms to survive. Cyanobacteria often dominate these cryoecosystems, but occupy restricted habitats such as under or within rocks, where they are physically protected to some degree from the harsh ambient conditions.

14.2.1 Lithic Environments

Lithobionthic microorganisms have been described at many sites in the Arctic and Antarctica, and occupy three types of habitat: *epilithic* organisms inhabit rock surfaces; *endolithic* organisms inhabit the rock interior (Fig. 14.2); *hypolithic* communities inhabiting the soil-rock interface, particularly where pebbles are translucent (Omelon 2008; Nienow and Friedmann 1993; Cockell and Stokes 2004). Within these different habitats the lithobionthic organisms can occupy different micro-regions: *euendolithic* micro-organisms can bore actively into the rock, and inhabiting the resultant hole (Cockell and Herrera 2007); *cryptoendolithic* micro-organisms inhabit the spaces between the grains of porous rocks (Omelon

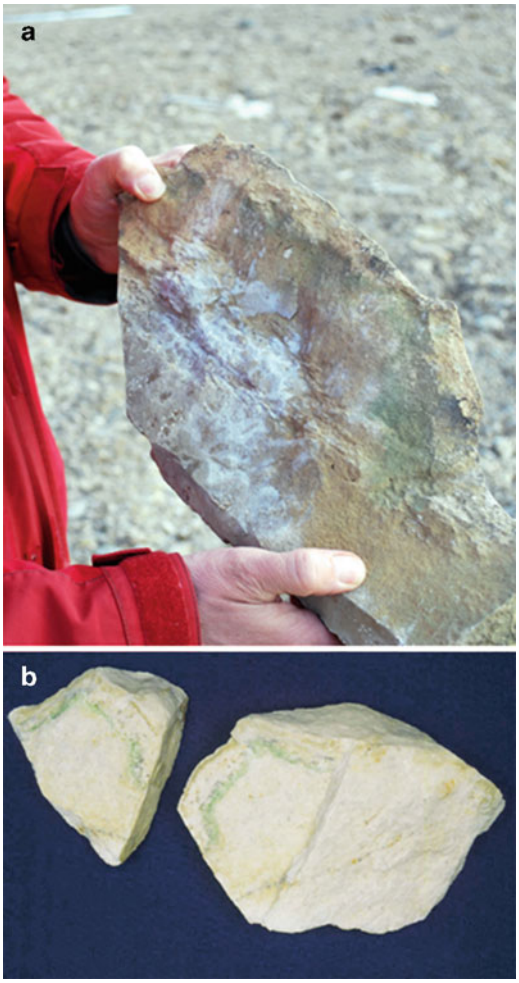

Fig. 14.2 Endolithic cyanobacteria in a sandstone rock from: (**a**) the High Arctic; and a quartz rock from: (**b**) the McMurdo Dry Valleys

et al. 2007); *chasmoendolithic* micro-organisms inhabit rock cracks and fissures (Büdel et al. 2008).

In the most severe polar desert environments such as the McMurdo Dry Valleys, few organisms can grow on exposed rock surfaces, but cyanobacteria and associated microbes are frequently found in endolithic or hypolithic environments. In less severe climates such as maritime Antarctica, epilithic communities are more frequent and diverse; conversely, in these habitats endoliths are rare, although also more diverse. Cyanobacteria are present in most polar cryptoendolithic polar habitats (Table 14.1), although they do not always dominate the total community biomass. The most abundant taxa belong to the orders Chroococcales: *Gloeocapsa* (several species), *Chroococcidiopsis*, *Aphanocapsa, Hormathonema*; and Oscillatoriales: *Lyngbya* and *Leptolyngbya*; and Nostocales: *Anabaena* (several species) and *Microchaete.* Some of these assemblages are conspicuously pigmented, such as the red *Gloeocapsa* community (Nienow and Friedmann 1993). The same genera are also found in chasmoendolithic communities, which are often dominated by *Chroococcidiopsis*, with associated populations of *Cyanothece* (Büdel et al. 2008). *Gloeocapsa* and *Chroococcidiopsis* also dominate Arctic hypolithic communities (Cockell and Stokes 2004). In coastal and maritime Antarctica these cyanobacterial taxa are accompanied by other genera such as *Merismopedia*, *Plectonema* and *Nostoc* (Nienow and Friedmann 1993).

Phototrophic lithobionts are limited by the amount of irradiance received within their sequestered habitats, and this restricts the rock type and depth of microbial penetration into the rock (Friedmann and Ocampo 1976). Most lithobiothic cyanobacteria require translucent rocks for colonization and growth. Primarily sandstone rocks (quartz) are colonized, but photolithobionthic communities are also found in evaporites (as gypsum) (Hughes and Lawley 2003), limestone and granite (De los Ríos et al. 2007). The irradiance transmission characteristics of the rock materials as well as those of the organisms growing inside the lithic environment result in a well marked stratification of the communities. Modelling analyses (Nienow et al. 1988) suggested that irradiance was 70–90% absorbed per mm of rock, although some mineral crusts can increase this attenuation very markedly. In polar deserts the availability of liquid water is a critical limiting factor for all organisms. Liquid water becomes available in endolithic ecosystems immediately after snow melt on the rock surface, although snowfall is infrequent. However, dew and rime can form in cold deserts such as the Taylor Valley (Antarctica) in much higher frequency than snowfall (Büdel et al. 2008). Once this liquid water is available for the endolithic community it can be retained there because of the slow evaporation due to reduced gas exchange with the atmosphere (Kappen et al. 1981), and desiccation tolerance is further aided by extracellular polysaccharides produced within the community (Knowles and Castenholz 2008).

Nitrogen availability does not seem to be a limiting factor for the phototrophic growth in endolithic habitats. Experiments by Vestal and coworkers (Johnston and Vestal 1986, 1991), adding combined nitrogen compounds (nitrate and ammonium) demonstrated that N probably was not limiting for the phototrophic organisms. On the other hand P can be limiting for this kind of community as shown by Banerjee et al. (2000) for three endolithic communities in sandstones from the Dry Valleys (Antarctica), where both the lithogenic P and the input from precipitation should be minimal. These authors demonstrated that organic P recycling via phosphatase activities plays a key role in the dynamics of the community. The nutrients used by the slow-growing endolithic community can be of the allochthonous origin (dust, nitrate deposition), of lithogenic origin and then recycling should play a crucial role in the nutrient dynamics.

Temperature is another factor that may often limit the activity of phototrophic organisms in polar habitats. Direct solar irradiance is the primary source of heat for the lithic communities. Under some circumstances the rock surface can heat to as much as 20°C above ambient air temperatures

Table 14.1 Dominant cyanobacterial genera described in cryospheric habitats, with examples of locations

Genus	Habitat	Location	References
Anabaena	Permafrost, Soil, Endolithic, Cryoconite	DV, Arctic, MA, AI	Friedmann et al. (1988) and Vishnivetskaya (2009) Mataloni et al. (2000) Brinkmann et al. (2007) Mueller and Pollard (2004) and Thompson (1989)
Aphanocapsa	Soil, Endolithic,	DV, MA	Friedmann et al. (1988) Fermani et al. (2007)
Aphanothece	Hypolithic, Chasmoendolithic Cryoconite	VH, MA, Arctic, DV	Broady (1981, 1986) Omelon et al. (2007) Mueller and Pollard (2004)
Calothrix	Soil, Hypolithic, Chasmoendolithic, Epilithic	MR, VH, ED, MA, VL	Broady (1981, 1986, 1989) Cavacini (2001)
Chamaesiphon	Soil, Epilithic, Cryoconite, Snow Lake Ice	MA, AI, DV, HM	Broady (1979) Priscu et al. (1998) Liu et al. (2006) Christner et al. (2003)
Chroococcidiopsis	Soil, Hypolithic Chasmoendolithic, Endolithic, Epilithic	MR, VH, DM, DV, ED, Arctic, MA	Broady (1981, 1986, 1989) Friedmann et al. (1988) Ryan et al. (1989) Cockell and Stokes (2004) Brinkmann et al. (2007) and Thompson (1989)
Cyanothece	Soil, Chasmoendolithic, Endolithic, Epilithic,	ED, MA, AI, DV	Broady (1989) Büdel et al. (2008) De los Ríos et al. (2004)
Eucapsis	Cryptoendolithic	DV	Friedmann et al. (1988)
Gloeocapsa	Soil, Hypolithic, Cryptoendolithic, Chasmoendolithic, Cryoconite	VH, DV, ED, Arctic, MA	Broady (1981, 1986, 1989) Friedmann et al. (1988) Cockell and Stokes (2004) Mueller and Pollard (2004)
Hormathonema	Soil, Cryptoendolithic,	DV	Friedmann et al. (1988)
Homoeothrix	Chasmoendolithic, Epilithic	MR, ED, MA	Broady (1981, 1989)
Leptolyngbya	Permafrost, Soil, Cryptoendolithic, Cryoconite, Glacial Ice, Lake Ice	Arctic, DV, MA, AI, VL	Vishnivetskaya (2009) and Friedmann et al. (1988) Cavacini (2001) Mataloni et al. (2000) Brinkmann et al. (2007) Priscu et al. (1998) Mueller and Pollard (2004) Stibal et al. (2006)
Lyngbya	Soil, Hypolithic Chasmoendolithic, Endolithic, Cryoconite	MR, DV, MA, VL, Arctic	Broady (1981) Friedmann et al. (1988) Mueller and Pollard (2004)
Microchaete	Soil, Endolithic	DV	Friedmann et al. (1988)
Microcoleus	Soil, Cryoconite, Glacial Ice	MA, AI, Arctic, DV	Komárek et al. (2008) and Brinkmann et al. (2007) Mueller and Pollard (2004) Stibal et al. (2006)
Myxosarcina	Epilithic	MA	Broady (1981)
Nodularia	Soil, Hypolithic, Chasmoendolithic,	VH, MA, VL	Broady (1981, 1986)

(continued)

Table 14.1 (continued)

Genus	Habitat	Location	References
Nostoc	Permafrost, Soil, Hypolithic, Chasmoendolithic, Epilithic, Cryoconite	MR, VH, DM, DV, ED, MA, AI, VL, Arctic	Broady (1981, 1986, 1989) Vishnivetskaya (2009) Büdel et al. (2008) Mueller and Pollard (2004) and Thompson (1989)
Oscillatoria	Soil, Endolithic, Cryoconite	VH, MA, Arctic, DV	Cameron (1972) Mataloni et al. (2000) Mueller and Pollard (2004)
Phormidium	Permafrost, Soil, Hypolithic, Chasmoendolithic, Endolithic, Epilithic, Cryoconite, Glacial Ice, Lake Ice,	DM, DV, ED, Arctic, MA. AI, VL	Ryan et al. (1989) Seaburg et al. (1979) Vishnivetskaya (2009) Priscu et al. (1998) Mueller and Pollard (2004) Stibal et al. (2006) and Thompson (1989)
Plectonema	Hypolithic, Chasmoendolithic	MR, VH, MA	Broady (1981, 1986) Nienow and Friedmann (1993)
Pleurocapsa	Hypolithic, Chasmoendolithic, Epilithic	VH, MA	Broady (1981, 1986)
Pseudanabaena	Soil	MA	Mataloni et al. (2000)
Schizothrix	Soil, Endolithic	DV	Cameron (1972)
Scytonema	Soil, Hypolithic, Epilithic,	DM, MA, VL	Ryan et al. (1989) Broady (1986) Cavacini (2001) and Thompson (1989)
Stigonema	Soil, Epilithic, Chasmoendolithic,	ED, MA	Broady (1989)
Synechococcus	Soil, Hypolithic, Cryptoendolithic, Epilithic, Cryoconite, Snow	DM, MA, AI, HM, Arctic, DV	Ryan et al. (1989) Broady (1979) Brinkmann et al. (2007) Liu et al. (2006) Omelon et al. (2007) Mueller and Pollard (2004) and Thompson (1989)
Tolypothrix	Soil, Hypolithic, Chasmoendolithic	VH, MA, AI	Broady (1979, 1981, 1986) Mataloni et al. (2000)

Locations: DV Dry Valleys, VH Vestfold Hills, MR Mawson Rocks, ED Edward VII Peninsula, DM Dronning Maud Land, MA Maritime Antarctica, AI Alexander Island, VL Victoria Land

(Omelon et al. 2006), thereby directly speeding up all biological activities but also thawing ice and thus increasing the liquid water availability. A factor distinguishing aquatic versus terrestrial ecosystems in the Polar Regions is the stability of the temperature regime. While in polar aquatic ecosystems temperature is usually low and relatively stable, in terrestrial habitats temperature fluctuates rapidly depending upon exposure to the sun; when these temperatures are close to freezing point, freezing and thawing processes can take place several times within few hours. For example, McKay and Friedmann (1985) measured in the McMurdo Dry Valleys a temperature fluctuation at the rock surface of 8°C over 42 min, crossing the freezing point 14 times. However, these rapid fluctuations are not expected or observed in the endolithic habitat due to the poor heat transmission of the rocks. Moreover, the abundant exopolysaccharides present in some of the cryptoendolithic communities increase the extent freezing tolerance (Knowles and Castenholz 2008).

The endolithic microbial communities show very low diversity but at least three trophic levels are typically included: primary producers (cyanobacteria, algae and lichens), consumers (fungi) and decomposers (heterotrophic bacteria). Walker and Pace (2007) have demonstrated, comparing the genetic identification of four endolithic communities from different parts of the world, that the communities

are quite similar, and present extremely low diversity, indicating also a high degree of microbial cosmopolitanism. However, Horath and Bachofen (2009) suggest that there is insufficient data to support the attributed simplicity and ubiquity of cyanobacteria in this kind of habitat. Some of the cyanobacteria found in endolithic habitat seem to be also present in other habitats as soils or semi-aerophytic environments (De los Ríos et al. 2007).

Endolithic communities represent the largest biomass (in organic C) compartment of Antarctic polar desert ecosystems, with organic carbon stocks of the order 10^2 g m^{-2} (Büdel et al. 2008; Nienow and Friedmann 1993), and with chlorophyll *a* contents in the range of tens of mg m^{-2}, which is only one order of magnitude lower than the values typically found in dense cyanobacterial mats in polar ponds (Chap. 13). It has been suggested that extremely low turnover rates take place within these microecosystems with an estimated age for lipids around 17,000 years (Vestal 1988; Johnston and Vestal 1991). Radiocarbon analyses in some endolithic communities indicate ages of the order of magnitude of 10^3 years (Bonani et al. 1988). However, the results presented by Büdel et al. (2008) indicate that these values depend very much on the water availability; the presence of the appropriate conditions for the dew formation may help to speed up all biological processes, reducing turnover rates to the order of hundreds of years. The cryptoendolithic community described by Büdel et al. in the granites of the Taylor Valley consisted of *Chroococcidiopsis*, *Cyanothece* and *Nostoc* and had a mean chlorophyll a content of 24 mg m^{-2} and an estimated mean biomass of 168 mg m^{-2}.

Endolithic environments may provide the most favourable terrestrial habitats for some cyanobacteria in extreme polar deserts, as indicated by the high cyanobacterial pigmentation and biomass sometimes observed below rock surfaces. Inside the rock, cyanobacteria may avoid many of the problems associated with the surface environment, with greatly improved liquid water availability, access to higher and more constant temperatures, protection against harmful UV radiation (Wynn-Williams 2000), and reduced habitat loss rates caused by wind erosion and rock surface detachment (Cockell and Herrera 2007).

14.2.2 Striped Ground and Polygonal Patterns

Hypolithic organisms are widely distributed in the Polar Regions, including areas of periglacial rock sorting (Thomas 2005). Hypolithic cyanobacteria are present under translucent rocks such as quartz (Cowan and Tow 2004), but also occur under opaque rocks such as dolomites (Cockell and Stokes 2006). They may represent an important source of biological activity and organic carbon for depauperate polar desert ecosystems. Cockell and Stokes (2004) have estimated the primary production of hypolithic cyanobacteria from High Arctic locations to be close to 1 g C m^{-2} $year^{-1}$, which is similar to the values for other phototrophs in these ecosystems. In hypolithic habitats, the sheltered environment beneath the rock provides a refugium, with much better microclimatic conditions than the upper rock surface. Liquid water availability seems to be one of the factors enhanced in the under-rock environment and one of the factors explaining the distribution of cyanobacteria in this habitat (Pointing et al. 2007). Rocks also protect organisms living underneath from high UVR (Cockell and Stokes 2006). Temperature changes are also very much attenuated, as in the endolithic habitat, providing a kind of greenhouse condition that is favourable for shade-adapted cyanobacteria (Wynn-Williams 2000). Intriguingly, hypolithic communities are especially common in patterned ground, where rock movements caused by the freeze-thaw cycles and the presence of ice in the soil provide a favourable combination of environmental conditions for cyanobacterial colonisation and growth (Cockell and Stokes 2004). On Devon and Cornwallis Islands (High Canadian Arctic) and on Alexander Island (Antarctica), 100% of the stones placed at the edges of the polygons in patterned grounds showed an evident hypolithic cyanobacterial community. However, the rocks inside the polygons showed much a lower colonization percentage (68% and 5% colonization in the Arctic and in the Antarctic, respectively). Conspicuous cyanobacterial biomass has been also described at the edge of large rocks at the perifery of frost sorted polygons (Hodgson et al. 2010).

Cyanobacteria growing in the hypolithic habitat apparently rest between lithic environments and edaphic (soil) habitats, however the genetic diversity of these organisms in the Dry Valleys indicated that they were more similar to aquatic and semi-aquatic cyanobacterial mats than to the soil microbiota (Wood et al. 2008). Morphologically the diversity of cyanobacteria in this habitat is quite poor, with *Chroococcidiopsis* the dominant genus, and *Gloeocapsa*-like cyanobacteria also frequent. However this morphological simplicity could be misleading. Pointing et al. (2007) working on non-polar hypoliths and comparing morphological observations and genetic tools suggested that some non-Chroococcales could adopt Chroococcales shapes in hypolithic ecosystems.

14.2.3 Soils

Soils may be the least hospitable habitat for cyanobacteria in the Polar Regions (Vincent 1988). Organisms inhabiting soil surfaces are exposed to bright solar radiation (including UV-A and UV-B), low humidity due to the wind effect, high amplitude fluctuations in temperature even within the same day, high salinity and osmotic stress, variable snow cover, and physical erosion and scouring associated with the intense winds that are frequent at high latitudes. In spite of all these limitations, phototrophic communities are often found in polar

soils, and cyanobacteria are usually the dominants (Vincent 1988; Fernández-Carazo et al. 2011; Michaud et al 2012). Edaphic cyanobacteria have been found at several sites around Antarctica and the Arctic (Vincent 1988), including polar deserts and more humid environments. However, it has been suggested that the presence of cyanobacteria in the most arid soils, such as those from the McMurdo Dry Valleys, is due to wind dispersion (Michaud et al. 2012) and that the soil populations are not actively growing (Aislabie et al. 2006). Consistent with this hypothesis, Wood et al. (2008) and Michaud et al. (2012) found that the cyanobacterial diversity in Dry Valley soils was very similar to that found in nearby microbial mats. Cyanobacteria from the three main orders (Chroococcales, Oscillatoriales and Nostocales) are frequently reported in Antarctic soils (Cavacini 2001; Wood et al. 2008). Humidity has been postulated as the main factor limiting the development of these edaphic cyanobacteria, but the chemical composition of soils may also play an important role, controlling the rate of supply of limiting elements and of toxic elements (Wood et al. 2008). Proximity to the inoculum source seems to be also a factor affecting the development of edaphic communities (Wood et al. 2008). Floristically, in most of the investigated soils the Oscillatoriales *Leptolyngbya* and *Phormidium* are the most common genera (Cavacini 2001; Mataloni et al. 2000). The occurrence of different cyanobacterial taxa seems to be related with the extent and duration of liquid water conditions (Vincent 1988). In mineral soils periodically flushed with water, Nostocales tend to dominate, but in moist to wet soils Oscillatoriales are the most frequent. Chroococcales are associated with other taxa in moist but unflushed sites. This distribution is related to the desiccation tolerance of each taxon, which is related to EPS concentration and characteristics (Wynn-Williams 2000). Yergeau et al. (2007) demonstrated in an Antarctic latitudinal gradient, extending from the subantarctic Falkland Islands (51°S) to the Ellsworth Mountain Range (79°S), that both diversity and species richness in fellfield microbial communities decreased as a function of increasing latitude. However, the proportion of cyanobacterial operational taxonomic units (OTU) was higher at higher latitudes (Yergeau et al. 2007). In the High Arctic (Svalvard, 78°N), 18 different cyanobacterial species were enumerated from barren soils, belonging to seven genera including *Leptolyngbya* and *Phormidium* (Kastovska et al. 2005).

Cyanobacteria are also abundant in high mountain environments. They become the dominant group in periglacial soils at 5,400 m altitude in Peru (Schmidt et al. 2009). The authors demonstrated that environmental conditions are even harsher than those in Polar Regions, with the temperature amplitude within 1 day reaching 36°C and with the fastest instantaneous cooling rate ever recorded of 1.83°C h^{-1}. Schmidt et al. (2009) identified a large number of cyanobacterial clones and a high diversity of genotypes that were only related to taxa from other high mountain sites and not to the Polar Regions.

Ancient communities of edaphic cyanobacteria have been preserved in permafrost. These cyanobacteria are not only fossil remnants from past ages, but in some cases they have been shown to be viable despite apparently being trapped and frozen in the permafrost for millions of years (Erokhina et al. 2000; Vishnivetskaya et al. 2002, 2003; Vishnivetskaya 2009). Several cyanobacterial strains belonging to the Oscillatoriales and Nostocales have been isolated from Arctic permafrost (Vishnivetskaya et al. 2003; Vishnivetskaya 2009) and show a close phylogenetic similarity to cyanobacteria found nowadays in microbial mats or endolithic environments, notably the genera *Leptolyngbya, Microcoleus, Phormidium, Nostoc* and *Anabaena*.

14.3 Ice-Based Ecosystems

Ice-based ecosystems have a number of characteristics that distinguish them from polar terrestrial ecosystems. One of the main differences is liquid water availability, which may be much greater in ice-based habitats as a result of radiative heating and local melting. Additionally, the environment is thermally more stable than in highly variable soil and rock regimes; temperatures are persistently cold, and changes are buffered by the isolating and high albedo characteristics of the ice and snow. Cyanobacteria occur in many types of ice-bound habitat, ranging from those that are annual or instable such as lake-ice (in lakes that melt out completely in summer) or snow, to those that are more stable in time, such as ice-shelves, glacier ice and cryoconites. The environmental conditions and the ecology of ice based ecosystems are described in detail in Laybourn-Parry et al. (2012).

14.3.1 Snow and Lake Ice

Cyanobacteria appear to be relatively rare in melting snow banks (Vincent 2000) or other non-stable cryoecosystems, probably because their slow growth is unable to keep pace with continuous losses by the percolating meltwater. However, they have been described in snow from glaciers in several areas of the world including Alaska, where two Oscillatoriales morphospecies were the only representatives (Takeuchi 2001), a Chilean Patagonia glacier, and in the Altai Mountains in Russia, where another Oscillatoriales morphospecies was found (Takeuchi and Koshima 2004; Takeuchi et al. 2006). On the Rombuk Glacier on Mount Everest, two groups of cyanobacteria were found in the snow at 6,500 m altitude, with genetic similarities to the Chroococcales genera *Synechococcus* and *Chamaesiphon* (Liu et al. 2006). In the Polar Regions, records for snow cyanobacteria are scarce and some authors such as Marshall and Chalmers (1997) suggest that are not actively growing in such environments, but merely transported and deposited on

snowpacks by the wind. The only report about Antarctic active cyanobacteria in snow is from an extensive snow cover on the Byers Peninsula (Livingston Island, maritime Antarctica) that was colonized by green algae, but also with *Leptolyngbya* as a substantial fraction of the biomass and several morphospecies of *Phormidium* (Velázquez et al. 2011). In the High Arctic, several cyanobacterial genotypes were found in the snow, and these were very similar to those found in the surrounding microbial mats (Harding et al. 2011).

Other transient cryo-ecosystems such as lake ice have not been investigated in detail, although in Antarctic lakes fragments of cyanobacterial mats wind blown are trapped during the ice formation and may remain active when they warm up due to solar radiation, even within the ice. In the perennial ice of the McMurdo Dry Valley lakes, cyanobacteria can remain for longer periods and be physiologically active (Priscu et al. 1998, 2005). Michaud et al (2012) identified a high cyanobacterial diversity from aeolian deposits in the McMurdo Dry Valleys as well as in the lake ice and suggested that these cyanobacteria can establish in the lake community when the liquid water is reached in summer partial melting.

Another example of unstable cryo-ecosystems is the annual sea-ice. In polar marine ecosystems cyanobacteria are not particularly abundant (Chap. 13), and there is little information regarding the occurrence of cyanobacteria in annual sea-ice. However, cyanobacteria have been recorded in multi-year Arctic sea-ice (Bowman et al. 2012).

14.3.2 Glacial Ice and Cryoconite Ecosystems

A cryoecosystem that is attracting increasing attention by microbial ecologists is the ice surface of glaciers where rock dust ('cryoconite') accumulates and initiates local melting. This material can build up to substantial levels, reaching values over 300 g m^{-2} for example on the Urumqi Glacier in the Tien Shan Mountains, China (Takeuchi and Li 2008). Many authors have found living cyanobacteria within these deposits (e.g. Takeuchi and Li 2008). This habitat provides favourable conditions for phototrophs, since the radiation absorption by the dust particles increases the surface temperature, causing the ice underneath to melt and release liquid water, which additionally may leach nutrients from the particles. The phototrophs are more abundant in the surface layers than in deeper layers indicating that their presence is not only a result of the dust deposition but also because of the active growth of these organisms (Xiang et al. 2009). This phototrophic growth and autochthonous carbon production also contributes towards the development of heterotrophic communities within the ice. Cyanobacteria have been described from polar glaciers (e.g. Mueller et al. 2001; Stibal et al. 2006) as well as from mountain glaciers around the world (Xiang et al. 2009). In an Arctic glacier the cyanobacterial communities found in supraglacial sediments (glacial kame) were dominated by *Leptolyngbya, Nostoc* and *Phormidium* (Stibal et al. 2006), while in high mountain glaciers the cyanobacterial communities were exclusively dominated by Oscillatoriales (Xiang et al. 2009).

More substantial melting of the glacier ice can give rise to cryoconite melt-holes consisting of cylindrical cavities, up to 50 cm deep, which retain dark sediment at the bottom and are filled with meltwater (Mueller et al. 2001). Cryoconite holes are abundant in the lower part of polar glaciers as well as on alpine glaciers of the temperate zone. In the cryoconite holes explored to date in Antarctica, cyanobacteria are the dominant biota, and their photosynthesis provides the basis for associated heterotrophic organisms, including a variety of micro-invertebrates as nematodes, rotifers and tardigrades (Mueller et al. 2001). Nutrient concentrations in the meltwater of the cryoconites are consistently low and phosphorus may be the limiting element (Mueller et al. 2001). The cyanobacterial community inhabiting cryoconites is quite similar all around the world, it is dominated by Oscillatoriales, notably *Leptolyngbya* spp. *Crinalium* spp., and *Phormidium* spp. Chroococcales are also abundant and typically represented by *Aphanothece, Aphanocapsa, Gloeocapsa* and *Synechococcus*. However, in most cryoconite holes in the White Glacier in the Canadian High Arctic, desmids rather than cyanobacteria dominated the community, possibly as a result of the lower pH of the meltwaters here (Mueller et al. 2001; Mueller and Pollard 2004). The cyanobacterial species distribution on cryoconites has been found to be quite similar to the species distribution in soils, ponds and mats in the proximities of the glaciers, indicating the wind distribution of these cryo-colonizers (Christner et al. 2003). The biological activity of primary producers in cryoconites has been described as a potentially significant important source of organic carbon to the polar environment (Säwström et al. 2002). However, in more dynamic glaciers, with shorter persistence of the cryoconites, autochthonous carbon input has been considered to be minor compared with the organic matter transported and accumulated in the cryoconite sediments (Stibal et al. 2008).

14.3.3 Ice Shelves

Ice shelves are sheets of ice from 10 to more than 100 m thick that are connected to land, but floating on the sea. They are found in coastal areas of both Polar Regions, but are vastly more extensive in Antarctica. The McMurdo Ice Shelf is the best known in Antarctica. It covers 1,500 km^2 and is one of the most spectacular ice-based ecosystems on Earth (Hawes et al. 2008). Sediment accumulates on the surface of this ice shelf by coming up from the sea bottom through the

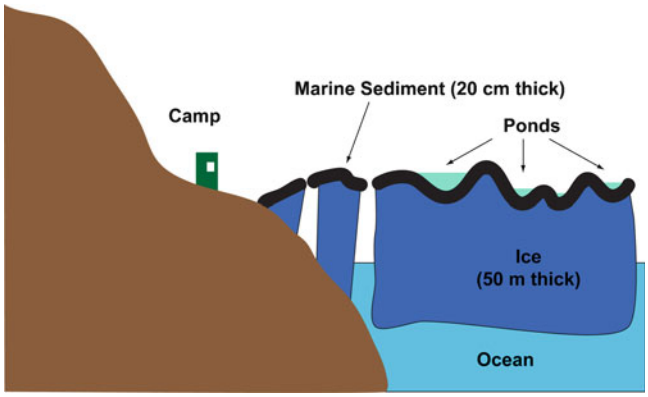

Fig. 14.3 Scheme of the McMurdo Ice-Shelf by Bratina Island. For clarity, drawings not to scale. Based on a field sketch by C. Howard-Williams

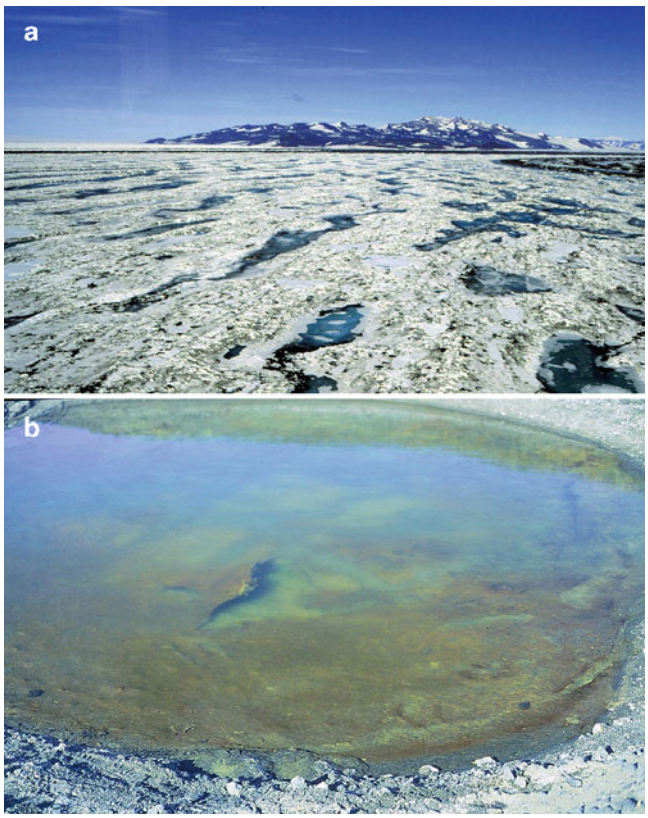

Fig. 14.4 McMurdo ice-shelf. (**a**) aerial photo showing the 'dirty ice', where numerous ice-based aquatic ecosystems are found; (**b**) detail of one of the ponds, showing cyanobacterial mats covering the *bottom*

basal accretion of ice, and the surface melting and ablation. This sediment forms a 10–20 cm thick layer (Fig. 14.3) that absorbs radiation and increases the temperature enough to melt the ice, producing ponds and wind-oriented lakes that can persist for decades or longer. In summer, when these lakes are ice-free in the surface, they function as traps for wind dispersed particles, including both sediments and inocula of new colonizers (Michaud et al. 2012). In the most stable ponds, cyanobacteria dominate the ecosystems forming extensive microbial mats, from mm to cm in thickness (Fig. 14.4). The microbial mats are formed by a matrix of *Leptolyngbya* (De los Ríos et al. 2004) accompanied by *Phormidium* and *Oscillatoria, Nostoc* and *Nodularia,* and several Chroococcales such as *Gloeocapsa*, *Synechococcus* and *Chroococcus*. Some green algae can also been found in the mats, as well as an associated diatom flora, the latter dominated by the genera *Navicula, Nitzschia, Pinnularia* and *Achnanthes* (Howard-Williams et al. 1990).

The microbial mats found in ice-shelf ponds and lakes are functionally similar to those found in other polar aquatic ecosystems (see Vincent and Quesada, this volume). Cyanobacterial N_2-fixation has been found responsible for an important proportion of the N input in the McMurdo Ice Shelf cryo-ecosystems, which seem to be N-limited (Fernández-Valiente et al. 2001).

Environmental conditions in the ice shelf ponds and lakes vary according to the type of sediment accumulated at a particular location. Thus, chemical diversity is as high as or even higher than in terrestrial based ponds and lakes. On the McMurdo Ice shelf, for instance, very low salinity ponds are found in very short proximity (hundreds of meters) to sulphate rich brines several times the salinity of sea water, and support cyanobacterial communities of different taxa (Vincent 2000; Vincent et al. 2004; Hawes et al. 2008).

In the High Arctic, ice shelves are restricted to the remnants of a continuous glacial fringe that at the beginning of the twentieth century occurred along 500 km of the northern coastline of Ellesmere Island, Canada. These remaining ice shelves are extremely vulnerable to climate change (Mueller et al. 2003). Over the period 2000–2008, they experienced considerable fracturing and attrition, including complete break-out and loss of the Ayles Ice Shelf and the Markham Ice Shelf (Vincent et al. 2009).

The High Arctic ice shelves have an undulating surface topography with localized deposits of sediments derived from three sources: wind-blown material; glacial moraine pushed onto the ice shelves by glaciers and other dynamic ice processes; and marine sediments that freeze into the bottom of the ice sheet and move to the surface by basal accretion of ice and surface ablation, as described above for the McMurdo Ice Shelf. Cyanobacterial mat consortia are found in association with the sediments, at the bottom of meltwater ponds or sometimes exposed to the air. Three types of mats can be distinguished in terms of visual appearance and community structure, and may represent a successional sequence (Mueller et al. 2006): sediments with little or no visible accumulation of microbial biomass, 'matlet' communities composed of loose flocs of olive-brown aggregates, and communities of accumulated matlets up to 10 mm thick, overlain by a thin (100 μm), more cohesive orange layer at the surface.

The dominant phototrophs in all three High Arctic communities are Oscillatoriales, particularly thin trichome representatives of *Leptolyngbya*, *Phormidium* and *Oscillatoria* (Mueller et al. 2006), as in Antarctica. However, nitrogen-fixing cyanobacteria are sparse relative to the McMurdo Ice Shelf communities, probably reflecting the low phosphorus concentrations and high N:P ratios in the High Arctic waters. The mats contain many chlorophytes, in particular *Palmellopsis, Chlorosarcinopis, Pleurastrum, Chlamydomonas Chlamydocapsa, Chlorella, Bracteacoccus, Chlorococcum*, and *Klebsormidium.* Like the McMurdo mats, these communities also contain abundant small benthic and aerophilic diatoms. The most common diatom species is *Chamaepinnularia* (*Navicula*) *begeri,* with subdominance by species of *Nitzschia, Navicula, Luticola, Achnanthes* and *Pinnularia* (Mueller et al. 2006).

Prior to its break-out in 2008, the Markham Ice Shelf contained the richest microbial mats, with extensive orange communities within and adjacent to meltwater ponds (Vincent et al. 2004). For this ice shelf alone, Mueller et al. (2006) estimated there to be 16,500 t microbial biomass in summer, with an additional 16,000 t on the much larger (and still extant in 2010) Ward Hunt Ice Shelf, and only sparse communities on the other four ice shelves. Analyses of these mats by high performance liquid chromatography (HPLC) showed that they are rich in UV photoprotecting pigments including scytonemin and many carotenoids (Chap. 13). Metagenomic analysis of these mats revealed that they contain a rich microbial flora in addition to cyanobacteria, with their ribosomal and protein-coding gene sequences dominated by Proteobacteria (Varin et al. 2010).

14.4 Cyanobacterial Diversity in the Cryosphere

Cyanobacterial diversity in the Polar Regions is a subject of ongoing debate. Some authors support the idea that several strains are endemic to Antarctica (Taton et al. 2006; Komárek et al. 2008). However, recently Jungblut et al. (2010) and Kleinteich et al. (2012) found that in cyanobacterial mats at least part of the genetic diversity was very similar to the genetic diversity found in Arctic and high mountain cyanobacterial mats, showing high 16S rRNA gene similarity (>99%) with some strains previously considered endemic to Antarctica. Morphological identification of polar cyanobacteria is complicated even at the genus level, and some genera have probably been misidentified. Strunecký et al. (2010) found that the very conspicuous genus *Phormidium* in polar regions can be misidentified and belong to several genera. They also found that strains isolated from both polar regions overlap geographically with ones isolated from temperate regions. This makes it almost impossible to compare the lists from different authors. Moreover, the genetic tools, although powerful in identifying genetic variation, are based on databases that are still very limited. Jungblut et al. (2010) concluded that, at least at the 16S rRNA gene level, cyanobacteria from polar and alpine regions are more related to each other than to temperate groups. However, they also noted the need for additional analyses using the ITS region (Comte et al. 2007), multilocus sequence analyses (Whitaker et al. 2003) and broader genomic and metagenomic analyses to define the ecotypic diversity of high latitude genotypes.

In Table 14.1 we present a non-exhaustive list of genera described in the cryosphere and the different habitats that they have been described from. It is especially remarkable that *Phormidium* has been found in the full range of habitats considered in this study, although it is also genus that can be easily misidentified. *Leptolyngbya* and *Nostoc* have been described from six different habitats, and as their morphological characteristics are easily distinguishable from other genera, most probably they have not been misidentified. These three genera can be considered cosmopolitan, at least within the cryosphere, since they appear in quite different habitats in terms of liquid water availability. At the other end of the range, *Chroococcidiopsis* seems to be especially well adapted to the habitats in which liquid water availability is limited, probably due to its extreme ability for desiccation resistance. It has even been suggested that it would be an ideal pioneer microorganism for terra-forming Mars (Friedmann and Ocampo-Friedmann 1995). On the other hand, *Microcoleus* seems to require the more frequent presence of liquid water (Table 14.1). Regarding the latitude at which they have been described, most genera have been found at maritime Antarctic sites, but also at coastal continental sites of Antarctica, with the associated contrasts in environmental conditions. The most common cyanobacterial genera appear to be present at all latitudes in the cryosphere where liquid water is available for some period each year.

14.5 Conclusions

Cyanobacteria are often the dominant organisms in cryoecosystems. This observation might seem paradoxical considering that most Arctic and Antarctic cyanobacteria studied to date are psychrotolerant rather than psychrophilic. Cryoecosystems are among the most extreme ecosystems on Earth, with liquid water limiting for most of the year, thermal conditions that can be highly unstable, and with rapid freeze-thaw cycles and associated fluctuations in water and salt stress. It is likely that these extremes preclude most other phototrophs, and allow cyanobacteria to achieve pre-eminence in these habitats. Cyanobacteria do not show high rates of metabolism and growth under these polar conditions, but

because of their broad tolerances rather than fine adaptation to cold, an ability to survive prolonged dormancy, and their resistance to natural loss processes, they are well suited to the cryosphere. Cyanobacteria in cryoecosystems appear to follow the 'lichen strategy' of remaining dormant and withstanding even the worst of environmental conditions, resuming primary production once conditions become suitable, and storing the new carbon, energy and perhaps even water for ongoing cell division. Psychrophilic adaptation would not be a suitable strategy in non-aquatic cryoecosystems, since high temperatures (>15°C) may be reached even in the coldest ice-free deserts, and the resultant thermal stress could be fatal to cold-adapted species. Furthermore, under these high temperatures, psychrotolerance rather than psychrotrophy would allow high biological activities to be achieved, and even a short period of enhanced performance each season may be enough to achieve a net positive energy and carbon balance. Cryptoendolithic cyanobacteria provide an extreme example of this strategy. These organisms cannot be considered ecologically successful using the traditional criteria of growth rates or metabolic performance, with impressively slow doubling times for the community biomass of hundreds or even thousands of years. Yet cyanobacteria are the most important primary producers in these habitats, until quite recently were considered to be devoid of life, and they have thereby achieved compelling success.

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References

Aislabie JM, Chhour KL, Saul DJ, Miyauchi S, Ayton J, Paetzold RF, Balks MR (2006) Dominant bacteria in soils of Marble Point and Wright Valley, Victoria Land, Antarctica. Soil Biol Biochem 38:3041–3056

Banerjee M, Whitton BA, Wynn-Williams DD (2000) Phosphatase activities of endolithic communities in rocks of the Antarctic Dry Valleys. Microb Ecol 39:80–91

Bonani G, Friedmann EI, Ocampo-Friedmann R, McKay CP, Woelfi W (1988) Preliminary report on radiocarbon dating of cryptoendolithic microorganisms. Polarforschung 58:199–200

Bowman JS, Rasmussen S, Blom N, Deming JW, Rysgaard S, Sicheritz-Ponten T (2012) Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. ISME J 6:11–20

Brinkmann M, Pearce DA, Convey P, Ott S (2007) The cyanobacterial community of polygon soils at an inland Antarctic nunatak. Polar Biol 30:1505–1511

Broady PA (1979) The terrestrial algae of Signy Island, South Orkney Islands, Scientific reports of the British Antarctic Survey 98. British Antarctic Survey, Cambridge

Broady PA (1981) Ecological and taxonomic observations on subaerial epilithic algae from Princess Elisabeth and MacRobertson Land, Antarctica. Br Phycol J 16:257–266

Broady PA (1986) Ecology and taxonomy of the Vestfold Hills. In: Pickard J (ed) Antarctic oasis: terrestrial environments and history of the Vestfold Hills. Academic, Sydney, pp 165–202, 367 pp

Broady PA (1989) Survey of algae and other terrestrial biota from Edward VII Peninsula, marie Byrd Land. Antarct Sci 1:215–224

Büdel B, Bendix J, Bicker FR, Green TGA (2008) Dewfall as a water source frequently activates the endolithic cyanobacterial communities in the granites of Taylor valley, Antarctica. J Phycol 44:1415–1424

Cameron RE (1972) Microbial and ecological investigations in Victoria Dry Valley, Southern Victoria Land, Antartcica. In: Llano GA (ed) Antarctic terrestrial biology. American Geophysical Union, Washington, DC, pp 195–260

Cavacini P (2001) Soil algae from northern Victoria Land (Antarctica). Polar Biosci 14:45–60

Christner BC, Kvitko BH II, Reeve JN (2003) Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole. Extremophiles 7:177–183

Cockell CS, Herrera A (2007) Why are some microorganisms boring? Trends Microbiol 16:101–106

Cockell CS, Stokes MD (2004) Widespread colonization by polar hypoliths. Nature 431:414

Cockell CS, Stokes MD (2006) Hypolithic colonization of opaque rocks in the Arctic and Antarctic polar desert. Arct Antarct Alp Res 38:335–342

Comte K, Sabacka M, Carre-Miouka A, Elster J, Komárek J (2007) Relationships between the Arctic and the Antarctic cyanobacteria; three *Phormidium*-like strains evaluated by a polyphasic approach. FEMS Microbiol Ecol 59:366–376

Cowan DA, Tow AL (2004) Endangered Antarctic environments. Ann Rev Microbiol 58:649–690

De los Ríos A, Wierzchos J, Sancho LG, Ascaso C (2004) Exploring the physiological state of continental Antarctic endolithic microorganisms by microscopy. FEMS Microbiol Ecol 50:143–152

De los Ríos A, Grube M, Sancho LG, Ascaso C (2007) Ultrastructural and genetic characteristics of endolithic cyanobacterial biofilms colonizing Antarctic granite rocks. FEMS Microbiol Ecol 59:386–395

Erokhina LG, Spirina EV, Shatilovich AV, Gilichinskii DA (2000) Chromatic adaptation of viable ancient cyanobacteria from arctic permafrost. Microbiology 69:855–856

Fermani P, Mataloni G, Van de Vijver B (2007) Soil microalgal communities on an active volcano (Deception Island, South Shetlands). Polar Biol 30:1381–1393

Fernández-Carazo R, Hodgson DA, Convey P & Wilmotte A (2011). Low cyanobacterial diversity in biotopes of the Transantarctic Mountains and Shackleton Range (80-82ºS), Antarctica. FEMS Microbiol Ecol 77:503–517

Fernández-Valiente E, Quesada A, Howard-Williams C, Hawes I (2001) N_2-fixation in cyanobacterial mats from ponds on the McMurdo Ice Shelf, Antarctica. Microb Ecol 42:338–349

Friedmann EI, Ocampo R (1976) Endolithic blue-green algae in the dry valleys: primary producers in the Antarctic desert ecosystem. Science 193:1247–1249

Friedmann EI, Ocampo-Friedmann R (1995) A primitive cyanobacterium as pioneer microorganism for terraforming Mars. Adv Space Res 15:243–246

Friedmann EI, Hua M, Ocampo-Friedmann R (1988) Cryptoendolithic lichen and cyanobacterial communities of the Ross desert, Antarctica. Polarforschung 58:251–259

Harding T, Jungblut AD, Lovejoy C, Vincent WF (2011) Microbes in high arctic snow and implications for the cold biosphere. Appl Environ Microbiol 77(10):3234–3243

Hawes I, Howard-Williams C, Fountain AG (2008) Ice-based freshwater ecosystems. In: Vincent WF, Laybourn-Parry J (eds) Polar lakes and rivers, limnology of Arctic and Antarctic aquatic ecosystems. Oxford University Press, New York, pp 103–108, 327 pp

Hodgson DA, Convey P, Verleyen E, Vyverman W, McInnes SJ, Sands CJ, Fernández-Carazo R, Wilmotte A, De Wever A, Peeters K, Tavernier I, Willems A (2010) The limnology and biology of the Dufek Massif, Transantarctic Mountains (82°S). Polar Sci 4:197–214

Horath T, Bachofen R (2009) Molecular characterization of an endolithic microbial community in dolomite rock in the Central Alps. Microb Ecol 58:290–306

Howard-Williams C, Pridmore R, Broady P, Vincent WF (1990) Environmental and biological variability in the McMurdo Ice-Shelf ecosystem. In: Kerry K, Hempel G (eds) Antarctic ecosystems. Ecological change and conservation. Springer, Berlin, pp 23–31, 427 pp

Hughes KA, Lawley B (2003) A novel Antarctic microbial endolithic community within gypsum crusts. Environ Microbiol 5:555–565

Johnston CG, Vestal JR (1986) Does iron inhibit cryptoendolithic communities? Antarct J US 21:225–226

Johnston CG, Vestal JR (1991) Photosynthetic carbon incorporation and turnover in Antarctic cryptoendolithic microbial communities: are they the slowest growing communities on Earth? Appl Environ Microbiol 58:2308–2311

Jungblut AD, Lovejoy C, Vincent WF (2010) Global distribution of cyanobacterial ecotypes in the cold biosphere. ISME J 4:191–202

Kappen L, Friedmann EI, Garty J (1981) Ecophysiology of lichens in the dry valleys of Southern Victoria Land, Antarctica. I. Microclimate of the cryptoendolithic lichen habitat. Flora 171:215–235

Kastovska K, Elster J, Stibal M, Santruckova H (2005) Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (high Arctic). Microb Ecol 50:396–407

Kleinteich J, Wood SA, Küpper FC, Camacho A, Quesada A, Frickey T, Dietrich DR (2012) Temperature-related changes in polar cyanobacterial mat diversity and toxin production. Nat Clim Change. doi:10.1038/nclimate1418

Knowles EJ, Castenholz RW (2008) Effect of exogenous extracellular polysaccharides on the desiccation and freezing tolerance of rock inhabiting phototrophic microorganisms. FEMS Microbiol Ecol 66:261–270

Komárek J, Elster J, Komárek O (2008) Diversity of the cyanobacterial microflora of the northern part of James Ross Island, NW Weddell Sea, Antarctica. Polar Biol 31:853–865

Laybourn-Parry J, Tranter M, Hodson AJ (2012) The ecology of snow and ice environments. Oxford University Press, Oxford 179 pp

Liu Y, Yao T, Kang S, Jiao N, Zeng YH, Shi Y, Luo TW, Jing ZF, Huang SJ (2006) Seasonal variation of snow microbial community structure in the East Rogbuk glacier, Mt. Everest. Chin Sci Bull 51:1476–1486

Marshall WA, Chalmers MO (1997) Airborne dispersal of Antarctic terrestrial algae and cyanobacteria. Ecography 20:585–594

Mataloni G, Tell G, Wynn-Williams DD (2000) Structure and diversity of soil algal communities from Cierva Point (Antarctic Peninsula). Polar Biol 23:205–211

McKay CP, Friedmann EI (1985) The cryptoendolithic microbial environment in the Antarctic cold desert: temperature variations in nature. Polar Biol 4:19–25

Michaud AB, Sabacka M, Priscu JC (2012) Cyanobacterial diversity across landscape units in a polar desert: Taylor Valley, Antarctica. FEMS Microbiol Ecol. doi:10.1111/j.1574-6941.2012.01297.x

Mueller DR, Pollard WH (2004) Gradient analysis of cryoconite ecosystems from two polar glaciers. Polar Biol 27:66–74

Mueller DR, Vincent WF, Pollard WH, Fritsen CH (2001) Glacial cryoconite ecosystems: a bipolar comparison of algal communities and habitats. Nova Hedwig 123:173–198

Mueller DR, Vincent WF, Jeffries MO (2003) Break-up of the largest Arctic ice shelf and associated loss of an epishelf lake. Geophys Res Lett 30:2031

Mueller DR, Vincent WF, Jeffries MO (2006) Environmental gradients, fragmented habitats, and microbiota of a northern ice shelf cryoecosystem, Ellesmere Island, Canada. Arct Antarct Alp Res 38:593–607

Nadeau T-L, Castenholz RW (2000) Characterization of psychrofilic Oscillatorians (Cyanobacteria) from Antarctic meltwater ponds. J Phycol 36:914–923

Nienow JA, Friedmann EI (1993) Terrestrial lithophytic (rock) communities. In: Friedmann EI (ed) Antarctic microbiology. Wiley-Liss, New York, pp 343–412, 634 pp

Nienow JA, McKay CP, Friedmann EI (1988) The cryptoendolithic microbial environment in the Ross Desert of Antarctica: mathematical models of the thermal regime. Microb Ecol 16:253–270

Omelon CR (2008) Endolithic microbial communities in polar desert habitats. Geomicrobiol J 25:404–414

Omelon CR, Pollard WH, Ferris FG (2006) Environmental controls on microbial colonization of high Arctic cryptoendolithic habitats. Polar Biol 30:19–29

Omelon CR, Pollard WH, Ferris FG (2007) Inorganic species distribution and microbial diversity within high arctic cryptoendolithic habitats. Microb Ecol 54:740–752

Pointing SB, Warren-Rhodes KA, Lacap DC, Rhodes KL, Mckay CP (2007) Hypolithic community shifts occur as a result of liquid water availability along environmental gradients in China's hot and cold hyperarid deserts. Environ Microbiol 9:414–424

Priscu JC, Fritsen CH, Adams EE, Giovannoni SJ, Paerl HW, McKay CP, Doran PT, Gordon DA, Lanoil BD, Pinckney JL (1998) Perennial Antarctic lake ice: an oasis for life in a polar desert. Science 280:2095–2098

Priscu JC, Fritsen CH, Paerl HW, Fritsen CH, Dore JE, Lisle JT, Wolf CF, Mikuchi JA (2005) Perennial Antarctic lake ice: a refuge for cyanobacteria in an extreme environment. In: Castello JD, Rogers SO (eds) Life in ancient ice. Princeton Press, Princeton, pp 22–49, 336 pp

Raymond JA, Fritsen CH (2000) Ice-active substances associated with Antarctic freshwater and terrestrial photosynthetic organisms. Antarct Sci 12:418–424

Ryan PG, Watkins BP, Smith RIL, Dastych H, Eicker R, Foissner W, Heatwole H, Miller WR (1989) Biological survey of Robertskollen, western Dronning Maud Land: area description and preliminary species list. S Afr J Antarct Res 19:10–20

Säwström C, Mumford P, Marshall W, Hodson A, Laybourn-Parry J (2002) The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79°N). Polar Biol 25:591–596

Schmidt SK, Nemergut DR, Miller AE, Freeman KR, King AJ, Seimon A (2009) Microbial activity and diversity during extreme freeze-thaw cycles in periglacial soils, 5400 m elevation, Cordillera Vilcanota, Perú. Extremophiles 13:807–816

Seaburg KG, Parker BC, Prescott GF, Whitford LA (1979) The algae of southern Victoria Land. A taxonomic and distributional study. J. Cramer Publishers, Stuttgart, 168 pp

Stibal M, Sabacká M, Kastovská K (2006) Microbial communities on glacier surfaces in Svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. Microb Ecol 52:644–654

Stibal M, Tranter M, Benning LG, Rehak J (2008) Microbial production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input. Environ Microbiol 10:2172–2178

Strunecký O, Elster J, Komárek J (2010) Phylogenetic relationships between geographically separate *Phormidium* cyanobacteria: is there a link between north and south polar regions? Polar Biol 33: 1419–1428

Takeuchi N (2001) The altitudinal distribution of snow algae on an Alaska glacier (Gulkana Glacier in the Alaska Range). Hydrol Process 15:3447–3459

Takeuchi N, Koshima S (2004) A snow algal community on Tyndall Glacier in the Southern Patagonia Icefield, Chile. Arct Antarct Alp Res 36:92–99

Takeuchi N, Li ZQ (2008) Characteristics of surface dust on Urumqi Glacier No.1 in the Tien Shan Mountains, China. Arct Antarct Alp Res 40:744–750

Takeuchi N, Uetake J, Fujita K et al (2006) A snow algal community on Akkem glacier in the Russian Altai mountains. Ann Glaciol 43:378–384
Tang EPY, Tremblay R, Vincent WF (1997) Cyanobacterial dominance of polar freshwater ecosystems: are high latitude mat-formers adapted to the low temperature environment? J Phycol 33:171–181
Taton A, Grubisic S, Brambilla E, De Wit R, Wilmotte A (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. Appl Environ Microbiol 69:5157–5169
Taton A, Grubisic S, Balthasart P, Hodgson DA, Laybourn-Parry J, Wilmotte A (2006) Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. FEMS Microbiol Ecol 57:272–289
Thomas DN (2005) Photosynthetic microbes in freezing deserts. Trends Microbiol 13:87–88
Thompson G (1989) Biological survey of Robertskollen, western Dronning Maud Land: area description and preliminary species list. S Afr J Antarct Res 19:10–20
Varin T, Lovejoy C, Jungblut AD, Vincent WA, Corbeil J (2010) Metagenomic profiling of Arctic microbial mat communities as nutrient scavenging and recycling systems. Limnol Oceanogr 55:1901–1911
Velázquez D, Rochera C, Camacho A, Quesada A (2011) Temperature effects on Antarctic non-marine phototrophic communities. Polar Biol 34:1045–1055
Vestal JR (1988) Carbon metabolism of the cryptoendolithic microbiota from the Antarctic desert. Appl Environ Microbiol 54:960–965
Vincent WF (1988) Microbial ecosystems of Antarctica. Cambridge University Press, Cambridge, 304 pp
Vincent WF (2000) Cyanobacterial dominance in the polar regions. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 321–340, 669 pp
Vincent WF (2007) Cold tolerance in cyanobacteria and life in the cryosphere. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Heidelberg, pp 289–304, 811 pp
Vincent WF, Howard-Williams C (2000) Life on snowball Earth. Science 287:2421
Vincent WF, Mueller DR, Bonilla S (2004) Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. Cryobiology 48:103–112
Vincent WF, Whyte LG, Lovejoy C, Greer CW, Laurion I, Suttle CA, Corbeil J, Mueller DR (2009) Arctic microbial ecosystems and impacts of extreme warming during the International Polar Year. Polar Sci 3:171–180
Vishnivetskaya TA (2009) Viable cyanobacteria and green algae from permafrost darkness. In: Margesin R (ed) Permafrost soils, vol 16, Soil biology. Springer, Heidelberg, pp 73–84, 348 pp
Vishnivetskaya TA, Vorobyova EA, Gilichinsky DA (2002) Viable green algae and cyanobacteria within terrestrial permafrost. In: Sawaya-Lacoste H (ed) Proceedings of the second European workshop on exo-astrobiology. ESA Publications Division, Noordwijk, pp 295–298, 625 pp
Vishnivetskaya TA, Spirina EV, Shatilovich AV, Erokhina LG, Vorobyova EA, Gilichinsky DA (2003) The resistance of viable permafrost algae to simulated environmental stresses: implications for astrobiology. Int J Astrobiol 2:171–177
Walker JJ, Pace NR (2007) Endolithic microbial ecosystems. Ann Rev Microbiol 61:331–347
Whitaker RJ, Grogan DW, Taylor JW (2003) Geographic barriers isolate endemic populations of hyperthermophilic archaea. Science 301:976–978
Wood SA, Rueckert A, Cowan DA, Cary SC (2008) Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica. ISME J 2:308–320
Wynn-Williams DD (2000) Cyanobacteria in deserts- life at the limit? In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 341–366, 669 pp
Xiang SR, Shang TC, Chen Y, Yao TD (2009) Deposition and postdeposition mechanisms as possible drivers of microbial population variability in glacier ice. FEMS Microbiol Ecol 70:165–176
Yergeau E, Newsham KK, Pearce DA, Kowalchuk GA (2007) Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. Environ Microbiol 9:2670–2682

Salts and Brines

15

Aharon Oren

Contents

A. Oren (✉)
Division of Microbial Molecular Ecology, Alexander Silverman Institute of Life Sciences and the Moshe Shilo Minerva Center for Marine Biogeochemistry, The Hebrew University 91904 Jerusalem, Israel
e-mail: orena@cc.huji.ac.il

Summary

Cyanobacteria form a major component of the biota of hypersaline environments including salt lakes, solar salterns, hypersaline lagoons, and hypersaline sulphur springs. Cyanobacteria are often found in evaporite crusts of gypsum and even halite. A wide range of species were reported to live at high salinities. In many hypersaline environments cyanobacteria are exposed to high sulphide concentrations. Certain species are able to use sulphide as an electron donor in an anoxygenic type of photosynthesis through a process which involves photosystem I only. To be able to withstand the high osmotic pressure caused by the salt concentrations in their surrounding medium, cyanobacteria living at high salinities possess mechanisms to maintain osmotic equilibrium and cell turgor. Ions can temporarily enter the cells to counteract rapid increases in medium salinity. For long-term osmotic stabilization organic solutes are accumulated: the disaccharides sucrose and trehalose, glucosylglycerol, and glycine betaine. Molecular analysis of glucosylglycerol metabolism in *Synechocystis* PCC 6803 has greatly increased our insight into osmoregulatory mechanisms. Understanding the molecular mechanisms of osmotic adaptation in cyanobacteria has led to the exploration of a number of interesting biotechnological applications.

15.1 Introduction

The cyanobacteria form a major component of the biota of salt lakes, solar salterns, hypersaline lagoons, salt flats, and hypersaline sulphur springs (Javor 1989; Oren 2002).

B.A. Whitton (ed.), *Ecology of Cyanobacteria II: Their Diversity in Space and Time*,
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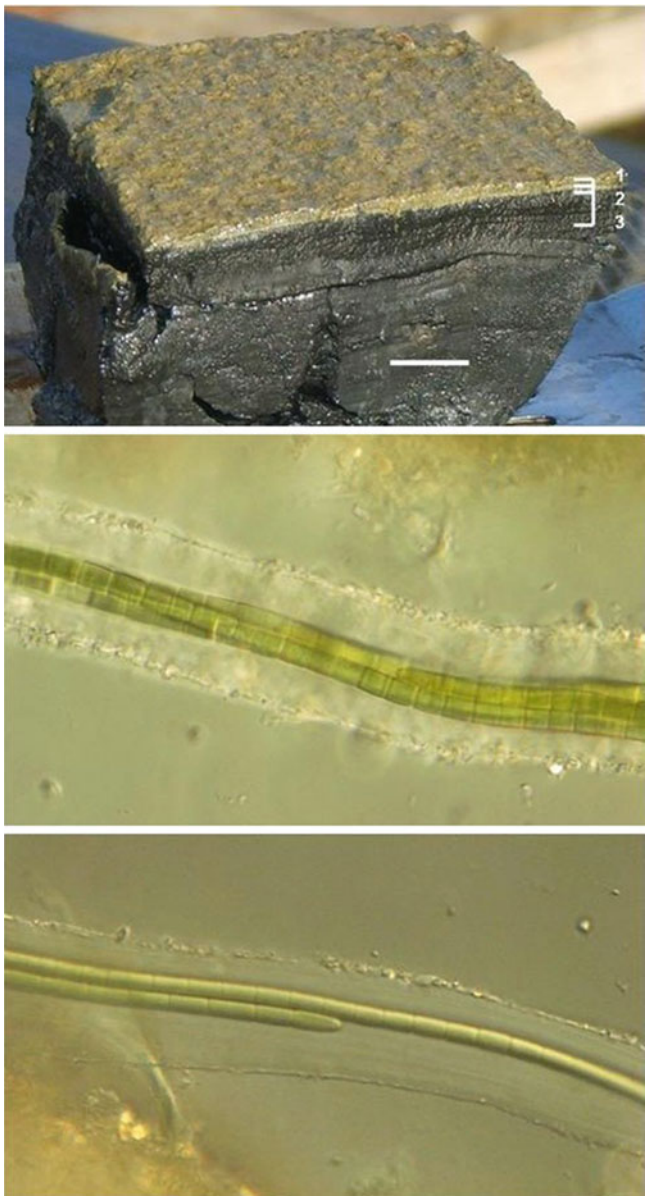

Fig. 15.1 The 'petola' mat in the crystallizer ponds of the salterns of Sečovlje, Slovenia (*upper panel*) and bundles of *Coleofasciculus chthonoplastes* (*Microcoleus chthonoplastes*) filaments within their polysaccharide sheaths

Dense communities of cyanobacteria are often a prominent feature of the biota in planktonic as well as in benthic environments at high salt concentrations. Light-exposed sediments in such environments are often covered with conspicuous thick mats of filamentous and unicellular cyanobacteria, which may be responsible for much of the primary production and thus form the basis for the functioning of the ecosystem (Bauld 1981; Des Marais 1995).

Cyanobacteria differ greatly in their relationship to salt. Many types are adapted to seawater salinities. The diversity of cyanobacterial life in the marine environment (around 35 $g\,l^{-1}$) and the physiology and the ecology of marine cyanobacteria are discussed in Chap. 4. Many of these cyanobacteria can also grow at higher salinities, making it virtually impossible to draw a sharp boundary between species adapted to the marine and to hypersaline habitats. Thus, *Microcoleus* (*Coleofasciculus*) *chthonoplastes*, the main mat building cyanobacterium in the marine littoral and intertidal flats, can be found in hypersaline environments worldwide up to salinities of 200 $g\,l^{-1}$ and higher (Dubinin et al. 1992a; Javor 1989), and is sometimes even found in sediments overlaying NaCl-saturated brine (Schneider and Herrmann 1980) (Fig. 15.1). A single, cosmopolitan form with the ability to adapt to a wide range of salinities appears to be present in all these environments (Garcia-Pichel et al. 1996). Several other types of cyanobacteria thrive at salinities close to NaCl (halite) saturation (Braithwaite and Whitton 1987; Rothschild et al. 1994). In a comparative study of salt tolerance and salt requirement, Golubic (1980) concluded that cyanobacteria speciate along the salinity gradient, and that separate halophilic taxa occupy environments with relatively constant salinities. He divided the cyanobacteria into stenohaline and euryhaline types (adapted to life within a narrow or a broad range of salt concentrations, respectively), and designated strains as oligo-, meso-, and polyhaline according to the optimal salt concentration for growth (for a definition of these and other related terms see Table 15.1).

In this chapter the boundary between "marine" and "hypersaline" systems is set arbitrarily at 70 $g\,l^{-1}$ salinity: twice the mean value of seawater. The chapter deals mostly with thalassohaline environments that originate from evaporation of seawater and thus reflect its ionic composition. A few cases of athalassohaline lakes (the Dead Sea, hypersaline soda lakes) with salt compositions greatly differing from that of ocean water are also discussed.

Table 15.1 Terms used in this chapter to describe salinity and salt composition of brines, and the response of microorganisms to high salt concentrations

Thalassohaline	Having an ionic composition resembling that of seawater
Athalassohaline	Having an ionic composition greatly differing from that of seawater
Halophilic	Salt-loving, requiring high salt concentrations for growth
Halotolerant	Being able to grow in the presence of high salt concentrations, but not requiring salt for growth
Stenohaline[a]	Adapted to life within a narrow range of salt concentrations
Euryhaline[a]	Adapted to life within a broad range of salt concentrations
Oligohaline[a]	Growing optimally at low salt concentrations
Mesohaline[a]	Growing optimally at intermediate salt concentrations
Polyhaline[a]	Growing optimally at high salt concentrations

[a]Terms derived from Golubic (1980)

15.2 Nomenclature Comments

Any treatise on cyanobacterial diversity is hampered by the poor state of cyanobacterial taxonomy and nomenclature, and discussions of cyanobacterial diversity at high salt concentrations are no exception. Synonyms abound, and different authors tend to use different designations for what may be the same (morphological) type. On the other hand, what may be referred to in different publications as the same genus and species may encompass a number different organisms. An additional problem is the existence of two nomenclature systems, the "botanical" system that follows the provisions of the International Code of Botanical Nomenclature, and the "bacteriological" system that follows the rules of the International Code of Nomenclature of Prokaryotes. These two nomenclature systems are in many respects incompatible (Oren and Tindall 2005). The (non-exhaustive lists) of genera and species that occur at high salt concentrations given by Borowitzka (1981), Golubic (1980), Oren (2002), and Potts (1980) contain mostly names described under the botanical system. Only very few names of cyanobacteria have been validly published under the bacteriological nomenclature system. One of these is the halophilic *Halospirulina tapeticola* (Nübel et al. 2000b).

In this chapter no attempt was made to adopt a uniform nomenclature system, and most species designations are those which appeared in the original reports cited. Here follow a few nomenclature comments referring to a number of species discussed in this chapter.

15.2.1 *Aphanothece halophytica*

The nomenclature of the unicellular exopolysaccharide-forming benthic (and sometimes planktonic) cyanobacteria found in many hypersaline environments is extremely confusing. The type most commonly known as *Aphanothece halophytica* Frémy is referred to in the literature also as *Coccochloris elabens, Synechococcus, Euhalothece,* and *Halothece*. Brock (1976) interchangeably used the names *Aphanothece halophytica* and *Aphanocapsa halophytica* for his strain from Great Salt Lake, Utah. The *Synechococcus* strain described from Abu Gabara Lake in Wadi Natrun, Egypt (Imhoff et al. 1979) and from salterns in Queensland, Australia (Coleman and White 1993) may belong to this group. The genus *Cyanothece*, created by Komárek (1976), includes part of the former genus *Synechococcus*. The strain isolated by De Philippis et al. (1993) from a salt pan in Somaliland and described as *Cyanothece* sp. was found to be very similar to an isolate from heliothermal salt works in Greece named *Cyanothece halobia* (Roussomoustakaki and Anagnostidis 1991), as well as an isolate from Solar Lake, Sinai, originally described as *Aphanothece shiloi* (Campbell and Golubic 1985) and later transferred to the genus *Cyanothece* as *Cyanothece shiloi* (Komárek and Anagnostidis 1995; see also Komárek and Cepák 1998). Borowitzka (1981) discussed the confusion in the earlier literature.

Phylogenetic analysis of a large collection of isolates of unicellular, extremely halotolerant cyanobacteria (Garcia-Pichel et al. 1998) showed that the group is diverse, but little was done to solve the nomenclature problem. The genus name *Euhalothece* was proposed at that time, but the name was never validly published (see Komárek et al. 2004). Later the genus name *Halothece* and species name *Halothece californiensis* were created for a group of morphologically somewhat different and phylogenetically distant strains, based both on a "botanical" and a "bacteriological" description of the newly proposed taxa (Margheri et al. 2008). The many problems created by this paper were exposed by Oren (2009b).

15.2.2 *Dactylococcopsis salina*

The name *Dactylococcopsis salina* was used to describe a long, spindle-shaped, gas-vacuolate halophilic cyanobacterium in Solar Lake, Sinai, and in saltern brines at different locations (Davis and Giordano 1996; Potts 1980; van Rijn and Cohen 1983; Walsby et al. 1983). Its cells are yellow to orange, and grow in culture between 5 and 20 $g\,l^{-1}$ salt (optimum 7.5–15 $g\,l^{-1}$) and up to 45°C. The genus name *Dactylococcopsis* has earlier been used for a genus of green algae, and therefore *D. salina* is an illegitimate name for a cyanobacterium. The name *Myxobactron salinum* is therefore more appropriate (Davis and Giordano 1996).

15.2.3 *Halospirulina tapeticola*

This type of highly halotolerant, euryhaline (30–200 $g\,l^{-1}$ salt), tightly coiled filaments tolerating elevated temperatures was classically assigned to the genus *Spirulina*. A phylogenetic study of a number of isolates led to a "bacteriological" description of *Halospirulina tapeticola* (Nübel et al. 2000b). It represents a rare case in which a name of a cyanobacterium was validly published according to the rules of the International Code of Nomenclature of Prokaryotes. According to Article 45(4) of the International Code of Botanical Nomenclature the name also has standing in the botanical nomenclature.

15.2.4 *Microcoleus chthonoplastes*

Phenotypic and phylogenetic analyses of isolates from marine and hypersaline environments worldwide showed

Microcoleus chthonoplastes to be a cosmopolite cyanobacterium (Garcia-Pichel et al. 1996). Its trichomes are generally encased in multiple-filament sheaths (Fig. 15.1). Filaments that may occur as individual trichomes may easily be mistaken for *Oscillatoria* or *Schizothrix* (Golubic 1980). Phylogenetically the halophilic species *M. chthonoplastes* and *M. steenstrupii* belong to the Phormidiaceae, while the freshwater species *M. vaginatus* belongs to the Oscillatoriaceae. Therefore reclassification of *M. chthonoplastes* was proposed as *Coleofasciculus chthonoplastes* (Thur. ex Gomont) Siegesmund *et al*. comb. nov. (Siegesmund et al. 2008). The far better known old name *M. chthonoplastes* is maintained below.

15.2.5 *Oscillatoria limnetica*

Under this name a cyanobacterium with about 3 μm wide filaments was described, isolated from Solar Lake, Sinai, that is capable of anoxygenic photosynthesis with sulphide as electron donor (Cohen et al. 1975b). The original description of *O. limnetica* Lemm. relates to a fresh water species with a much smaller filament width. The Solar Lake strain was later described as *Phormidium hypolimneticum* (Campbell and Golubic 1985; Golubic 1980).

15.2.6 *Halomicronema excentricum*

The description of *Halomicronema excentricum*, a halophilic non-heterocystous thin filamentous cyanobacterium (Abed et al. 2002) neither conforms the demands of the International Code of Botanical Nomenclature (no Latin diagnosis, no non-living type specimen designated) nor those of the International Code of Nomenclature of Prokaryotes (valid publication of the name based on a type strain deposited in at least two culture collections located in different countries), so that the name has no standing under either nomenclature code.

15.3 Hypersaline Environments and Their Cyanobacterial Communities

This section presents an overview of the types of cyanobacteria and their activities in a variety of high-salt environments: hypersaline lakes, sulphur springs, hypersaline lagoons and salt flats, and man-made salterns for the production of salt from seawater. As expected when environmental conditions become more adverse, the higher the salt concentration, the lower the diversity of cyanobacteria encountered. Cyanobacteria are not the most salt-tolerant of all oxygenic phototrophs: the unicellular green alga *Dunaliella salina* dominates thalassohaline environments up to halite saturation, and primary production in the Dead Sea is also due to *Dunaliella* and not to cyanobacteria (Oren and Seckbach 2001; Seckbach and Oren 2007). Only rarely are cyanobacteria found to be active at such extremely high salinities (for exceptions see e.g. Braithwaite and Whitton 1987; Rothschild et al. 1994; Schneider and Herrmann 1980).

Cyanobacteria found in hypersaline environments are almost invariably of the unicellular type or unbranched non-heterocystous straight or coiled filaments. Branched filamentous forms are lacking altogether, and heterocystous species were seldom recorded. A salinity of 70 $g\,l^{-1}$ appears to be upper limit for nitrogen fixation by *Nodularia spumigena* in Farmington Bay, the south arm Great Salt Lake, Utah (Marcarelli et al. 2006). Heterocystous *Scytonema* was found in Storr's Lake, San Salvador Island, Bahama Islands at 45–>90 $g\,l^{-1}$ salt (Paerl et al. 2000). Nitrogen fixation can proceed at higher salinities by non-heterocystous cyanobacteria at higher salinities, as studies in coastal pools of the Sinai Peninsula and in the hypersaline mats of Guerrero Negro, Baja California have shown (Potts 1980; Rothschild et al. 1994).

15.3.1 Hypersaline Lakes

15.3.1.1 Great Salt Lake, Utah

Great Salt Lake, Utah, is a large inland lake. In spite of the fact that no direct connection with the open ocean has existed for tens of thousands of years, the ionic composition of its waters is very similar to that of seawater. The overall salinity of the lake was subject to drastic changes during the past decades. In 1959 the lake was divided by means of a causeway, and this led to the formation of a hypersaline northern arm (Gilbert Bay, total salt concentration up to 250–300 $g\,l^{-1}$ and higher) and a less saline southern arm (around 120 $g\,l^{-1}$ in the 1970s) (Post 1977). A large excess of rainfall in the late 1980s-early 1990s in the catchment area of the lake caused a sharp drop in overall salinity. Currently (2008) the northern arm has an average salinity of around 270 $g\,l^{-1}$ and the southern arm contains 60–100 $g\,l^{-1}$ only.

The older studies on the microbiology of Great Salt Lake were reviewed by Post (1977, 1981). While most of the primary production in the lake is due to eukaryotic unicellular algae (*Dunaliella* spp.), cyanobacteria are a characteristic component of the lake's biota. *Aphanothece halophytica* was found at the highest salinities. Brock (1976) recognized the existence of halophilic cyanobacteria based on the occurrence of *Aphanothece* in Great Salt Lake and on the properties of an isolated strain in culture. In addition, species of *Phormidium* or *Oscillatoria*, as well as *Microcoleus lyngbyaceus*, *Spirulina major,* and *Nodularia spumigena* were

found in the shallow sediments of the lake (Felix and Rushforth 1979; Post 1977, 1981; Rushforth and Felix 1982).

Currently *Nodularia spumigena* is the dominant cyanobacterium in the less-saline southern arm, where it contributes to nitrogen fixation (Marcarelli et al. 2006; Roney et al. 2009). It is accompanied by *Oscillatoria* and *Phormidium* sp. and sometimes by *Spirulina* sp. (identified as *S. labyrinthiformis*). In the near-salt-saturated northern basin the only cyanobacterium found is *A. halophytica* (Roney et al. 2009).

15.3.1.2 The Dead Sea

The Dead Sea is an athalassohaline inland lake on the border between Israel and Jordan. Its extremely saline waters (currently around 347 $g\,l^{-1}$ total dissolved salts) have an unusual ionic composition: presently the lake contains about 1.98 M Mg^{2+} and 0.42 M Ca^{2+}, in addition to about 1.54 M Na^{+} and 0.21 M K^{+} (2007 values). The dominant anion is Cl^{-}, followed by Br^{-} (about 1% of the anion fraction). The pH of the brine is around 6.

The microorganisms best adapted to this hostile environment are species of halophilic Archaea and one primary producer, the unicellular green alga *Dunaliella parva*, and even these do not grow in undiluted Dead Sea water. Only when winter floods of fresh water have significantly diluted the upper water layers do microbial blooms develop (Oren 1988, 2002).

Cyanobacteria do not form an important part of the Dead Sea ecosystem. Strains identified as *Aphanothece, Microcystis, Phormidium* and *Nostoc*, all developing at salt concentrations of up to 180 $g\,l^{-1}$, were obtained in enrichment cultures set up in a period when the salinity of the surface waters was about 21% less than today (Volcani 1944). One obligately halophilic *Aphanocapsa* strain was reported to grow at salt concentrations between 60 and 360 $g\,l^{-1}$. The isolate changed its morphology in response of the salt concentration of the medium. Small cells, singly or in pairs, occurred at the low salinity range and large rounded and vacuolate cells were present at the highest salinities (Volcani 1944). No quantitative data are available about the microbial communities in the lake during that period and the possible contribution of cyanobacteria to its biota. No cyanobacteria were ever detected by direct microscopic examination of Dead Sea water or sediment samples.

15.3.1.3 Solar Lake, Sinai

Solar Lake is a small hypersaline heliothermal lake on the shore of the Sinai coast, Egypt. In summer, the lake (maximum depth 4.5–5 m) is mixed, with a uniform salinity of ~180 $g\,l^{-1}$. Seepage of seawater from the Gulf of Aqaba (41 $g\,l^{-1}$ salt) and occasional rain floods cause the formation of a less saline layer (as low as 68 $g\,l^{-1}$) in autumn – winter, and the lake remains stratified from October until May. During stratification a chemocline separates an anaerobic sulphide-containing hypolimnion from the oxidized epilimnion. The difference in density of the upper less saline 1–2 m of the water column and the deep hypersaline brines prevents mixing, and heliothermal heating causes the hypolimnion to reach maximal temperatures of up to 49–61°C in winter. A detailed account of the limnology of the lake was given by Cohen et al. (1975a, 1977a).

The microbiology of Solar Lake has been intensively studied in the 1970s and 1980s. A rich cyanobacterial flora was found both in the water column and in the benthic microbial mats. The list of species found included *Aphanocapsa concharum, Aphanothece stagnina, Aphanothece microscopica, Aphanothece halophytica, Aphanothece littoralis, Aphanothece pallida, Chroococcidiopsis* sp., *Cyanothece* (*Aphanothece*) *shiloi, Dactylococcopsis salina, Dactylococcopsis acicularis, Entophysalis* sp., *Gloeothece* sp., *Johannesbaptistia pellucida, Lyngbya aestuarii, Lyngbya confervoides, Lyngbya diguetii, Microcoleus chthonoplastes, Oscillatoria redekei, Oscillatoria limnetica, Oscillatoria salina, Phormidium hypersalinum, Phormidium hypolimneticum, Pleurocapsa* sp., *Pseudanabaena catenata, Spirulina labyrinthiformis,* and *Synechococcus* sp. (Ali 1999; Campbell and Golubic 1985; Cohen et al. 1975a, 1977b; Jørgensen et al. 1983; Potts 1980). The mixed water column during the summer months contained *Aphanothece* cells at a low density. *Aphanothece* was also found in the upper water layer during winter stratification. At the border between the aerobic epilimnion and the hot, anaerobic hypolimnion a population of *Dactylococcopsis salina* (designated *Myxobactron salinum* by Davis and Giordano 1996; see also Sect. 15.2) was found (Potts 1980; van Rijn and Cohen 1983; Walsby et al. 1983). Its yellow to orange coloured gas-vacuolate cells grow in culture between 5 and 20 $g\,l^{-1}$ salt (optimum 7.5–15 $g\,l^{-1}$) and up to 45°C. The hot, sulphide containing water layer at the bottom of the lake contains a dense population of *Oscillatoria limnetica* (*Phormidium hypolimneticum*; see Campbell and Golubic 1985) which grows anaerobically, using sulphide as electron donor in an anoxygenic type of photosynthesis (see Sect. 15.5).

Different types of benthic cyanobacterial mats can be found at Solar Lake. The surface mats (different mat morphologies being described as "shallow flat mat", "deep flat mat" and "blister mat", all at salt concentrations around 80 $g\,l^{-1}$ and temperatures between 25°C and 30°C) were dominated by *Microcoleus, Phormidium, Aphanothece, Aphanocapsa,* and *Synechococcus*. Nitrogen fixation as measured by the acetylene reduction assay occurred in the *Microcoleus*-dominated mats of Solar Lake (Potts 1980), although *Microcoleus* itself was never shown to fix nitrogen. The brownish-red summer mat was dominated by *Aphanothece halophytica* and *Aphanothece littoralis* (Cohen 1984a; Jørgensen et al. 1983; Krumbein et al. 1977; Revsbech et al. 1985). The peripheral crust around the pool contained

Pseudanabaena, and the gypsum crust close to water was inhabited by *Entophysalis* (Potts 1980). *Lyngbya aestuarii* appeared as discontinuous patchy films over the surface of Solar Lake mats (Potts 1980). An approximately 10 mm thick gelatinous, polysaccharide-rich mat was found in winter in the thermocline area (salinity about 150 g l^{-1}, temperature around 45°C). This mat lacked *Microcoleus* and was rich in *Phormidium, A. halophytica, Aphanocapsa* and *Pleurocapsa*, which imparted a bright orange color to the upper 7 mm of the mat. Below this depth the color changed to light green due to the presence of *Oscillatoria* sp., accompanied by *A. halophytica*. Rates of gross and net photosynthesis in the mats were optimal around 30°C (Wieland and Kühl 2000).

15.3.1.4 Other Hypersaline Lakes

Several other hypersaline lakes in different parts of the globe have been explored for planktonic and benthic cyanobacteria. Below are a few examples:

- A variety of inland lakes are found on the Australian continent, with salinities from 100 g l^{-1} to almost 300 g l^{-1}. Different types of filamentous (*Microcoleus, Phormidium, Spirulina, Oscillatoria*) and unidentified unicellular cyanobacteria, were found in these environments (Bauld 1981).
- A 5–10 mm thick mucilaginous cyanobacterial mat with *Cyanothece, Oscillatoria*, and *Spirulina* species was found in Lake Hayward, South-West Australia, a hypersaline, seasonally stratified lake with salt concentrations varying from 60 g l^{-1} in the upper water layer to 200 g l^{-1} at the bottom (Burke 1995).
- Hot Lake, Washington, a stratified lake with 100 g l^{-1} total dissolved salts in the surface layer, increasing to 400 g l^{-1} near the bottom, was found to be inhabited by *Anacystis thermalis, Gomphosphaeria aponina, Oscillatoria chlorina*, and *Plectonema nostocorum* (Anderson 1958). No later studies appear to have been reported on the microbiology of this unusual environment.
- Salt Pond, San Salvador Island, Bahamas, ranges in salinity between 114 and 104 g l^{-1} in the dry season (March) to 87 g l^{-1} in the wet season (October). Molecular 16S rRNA gene-based studies showed presence of diffent types of Chroococcales and Oscillatoriales, with Pleurocapsales and Nostocales as minor components. Sequences of *nifH* related to the Oscillatoriales and unicellular cyanobacteria were also found (Yannarell et al. 2000). The properties of the cyanobacterial mats in this lake were investigated as indicators of elevated tropical hurricane activity and the associated climate change. Freshwater input causes a surge in activity of the mats consisting of *Microcoleus, Lyngbya, Schizothrix, Oscillatoria* and *Johannesbaptistia*. Growth of *Microcoleus* and *Lyngbya* was especially stimulated following hurricane rain floods (Paerl et al. 2003).
- Lake Salada de Chiprana, NE Spain, is a 5.6 m deep inland lake with a salinity of 78 g l^{-1}. The bottom is covered by a mat consisting of *Microcoleus* as well as *Pseudanabaena*-like filamentous and *Aphanothece* and *Gloeocapsa*-like unicellular cyanobacteria. A study was made of the depth distribution of chlorophyll *a* and three of its allomers (Chla_1, Chla_2, Chla_3). The different allomers may be functional adaptations to differences in light quality and/or quantity. They showed diel changes in vertical distribution due to migration of the cyanobacteria harboring these pigments (Jonkers et al. 2003). A study of nutrient enrichment in mesocosms showed that addition of phosphate stimulated growth of *Microcoleus* as well as nitrogen fixation (not due to *Microcoleus* as this organism does not have nitrogenase; Camacho and de Wit 2003).
- A *Cyanobium*-like cyanobacterium was isolated from the hypersaline (95 g l^{-1} salt) and alkaline (pH 9.8) Mono Lake, California. Gene sequences corresponding with those of the ribulosebisphosphate carboxylase/oxygenase (RUBISCO) gene of this organism were recovered from DNA isolated from the planktonic biomass (Giri et al. 2004).
- Lakes and pools of the Wadi Natrun, Egypt, are characterized by high salinities accompanied by high pH values (10.8–11.2). The microbiology of these lakes was described by Imhoff et al. (1979). Unspecified cyanobacteria were reported to occur in lakes Gaar, Rizunia, Zugm, and Muluk (374, 389, 394, and 159 g l^{-1} salts, respectively). Lake Gabara (91.9 g l^{-1}) contained *Spirulina* sp., and a strain of *Synechococcus* was isolated from Lake Hamra (240 g l^{-1} salt).
- Lake Bonney in the Dry Valleys of Antarctica has benthic mats dominated by *Lyngbya martensiana* and *Phormidium frigidum*, occurring at a salt concentration of about 100 g l^{-1} (Wharton et al. 1983).
- Lake Vanda in Wright Valley, Antarctica, was found to contain coccoid (*Synechococcus* sp.) and filamentous cyanobacteria (*Phormidium* type) (about 10^6 cells and 10^5 filaments l^{-1}, respectively, at 60 m depth at a salinity of about 100 g l^{-1}) (Goldman et al. 1967).
- Don Juan Pond in Antarctica is a hypersaline pond, with salt concentrations (mainly $CaCl_2$) reported to vary between 200 and 474 g l^{-1}. *Oscillatoria*-like cyanobacteria were observed as a mat near the shore of the lake (Siegel et al. 1979). This growth probably occurred during inundation by ephemeral freshwater meltstreams rather than in the concentrated $CaCl_2$ brines of the pond itself (Wright and Burton 1981).

15.3.2 Hypersaline Sulphur Springs

The springs of Hamei Mazor, that were exposed on the western shore of the Dead Sea in the 1980s and early 1990s were

characterized by a high salt content (around 170 g l^{-1} total dissolved salts), a high sulphide con concentration (about 2.5 mM), and a pH of 5.2. The temperature of the water at the source was 39°C. The pools and runoff channels formed by the spring were covered by a layer of filamentous cyanobacteria (*Phormidium* sp.), accompanied by patches of *Thiocapsa*-like purple sulphur bacteria. Photoassimilation of CO_2 by the cyanobacterial community was not inhibited by 3(3,4-dichlorophenyl)1,1-dimethylurea (DCMU), and the presence of sulphur granules associated with the cyanobacterial filaments suggested that photosynthesis is of the anoxygenic type, with sulphide as electron donor (Oren 1989; see also on Sect. 15.5). As a result of hydrological changes in the Dead Sea area these interesting springs have dried out, and the cyanobacteria-covered streams of hypersaline sulphidic water are no longer present.

15.3.3 Hypersaline Lagoons and Salt Flats

In marine coastal environments, especially in arid areas of the tropics and subtropics, shallow areas covered with seawater often become partially isolated from the sea. Along the coast of the Sinai Peninsula, the Arabian Gulf and elsewhere, salt flats, the so-called sabkhas, are formed when seawater-covered area are isolated from the open sea, but still receive a slow exchange of water through a separating sand bar. Evaporation rates exceed the rate of water inflow and cause an increase in salinity, until a more or less steady-state salinity is reached which exceeds that of seawater.

Extensive studies were made of the cyanobacterial communities that inhabit different hypersaline environments on the coast of the Sinai Peninsula (Egypt): Solar Lake (discussed above), a former sabkha near Nabq (at the time known also as Sabhka Gavish; now a built area), and the Ras Muhammad Pool. All receive a supply of seawater through subterranean seepages (Cohen et al. 1975a, 1977a; Ehrlich and Dor 1985; Gerdes et al. 1985; Javor 1989; Potts 1980). In total, 41 cyanobacterial species were recorded from the hypersaline coastal pools, including 24 in Solar Lake and 13 from the Ras Mohammed Pool (Javor 1989; Potts 1980). A clear horizontal zonation of species was reported in the sabkhas. The peripheral zone of the Gavish sabkha and its gypsum crust was dominated by *Pleurocapsa* and *Entophysalis*. The *Pleurocapsa*-inhabited gypsum nodules showed nitrogen fixation (acetylene reduction) activity. Towards the center a red-orange zone of *Aphanothece*, accompanied by *Synechococcus*-type unicellular cyanobacteria was found, while the central area was covered by a green mat of *Microcoleus* together with *Oscillatoria* and *Lyngbya* spp. (Potts 1980). An additional inhabitant was *Spirulina subsalsa*, found up to a salinity of about 205 g l^{-1}. Only two species were found at the highest salinities (250–330 g l^{-1}): *Aphanothece halophytica* and *Schizothrix arenaria* (Ehrlich and Dor 1985; Gerdes et al. 1985).

Recently studies were made of the microbial mats across intertidal flats of the arid coast of the Arabian Gulf (Abu Dhabi, United Arab Emirates), with special emphasis on lipid biomarkers, pigments and cyanobacterial diversity. Increased amounts of unsaturated fatty acids (12–39% of the total) and increased *trans/cis* ratio (0.6–1.6%) of the n-18:1 Δ^9 fatty acid in the salt flats located at the higher and drier area suggest adaptation of the mat microorganisms to environmental stress caused by elevated temperatures (up to 50°C in summer) and salinities (up to 200 g l^{-1}, compared to 60 g l^{-1} and 80–105 g l^{-1}) in the low and middle intertidal areas. *Microcoleus* and *Lyngbya aestuarii* were the dominant morphotypes; *Halothece*-like cyanobacteria were present in the deeper layers (Abed et al. 2006, 2008). Microelectrode measurements showed maximum photosynthesis rates at 45°C. Vertical diel migration of the *Microcoleus* filaments was observed, and the cells probably shifted to anoxygenic photosynthesis at the higher temperatures when sulphide started to accumulate (Abed et al. 2006).

In the lagoons of Lake Sivash (east Crimea, Ukraine), *Microcoleus* mats are the main primary producers at salt concentrations between 80 and 160 g l^{-1} (Zavarzin et al. 1993), and are even found up to 300 g l^{-1} (Zvyagintseva et al. 1995).

Most of the salt flats of Guerrero Negro (Baja California Sur, Mexico) are part of the Exportadora de Sal evaporation ponds, and these are discussed in the next section. Natural salt flats also occur outside the salina area, and these were also the subject of study (Rothschild et al. 1994; Stolz 1990). Evaporite crusts of gypsum and halite are formed, up to 4 cm or more in thickness, and often display distinct colorful layers of unicellular and filamentous cyanobacteria. The cyanobacteria that inhabit these dry crusts may remain active at least for many months after their formation (Rothschild et al. 1994).

The Great Salt Plains, Oklahoma, form an interesting inland hypersaline environment, with pools of salinities of 100 g l^{-1} and higher, as well as dry salt crusts. Cyanobacterial diversity and halotolerance was studied in this variable hypersaline environment. The genus *Phormidium* and its subgenus *Geiterinema* were most abundant. A number of environmental 16S rRNA sequences retrieved belonged to heterocystous lineages. Isolates obtained from the area included colonial Chroococcales (*Aphanothece*/*Aphanocapsa*), a Nostocalean strain, *Chlorogloeopsis*, and *Nodularia*. Most isolates were halotolerant up to 50 g l^{-1} NaCl only, but some could grow up to 150 g l^{-1}. One *Geitlerinema* strain grew at a wide range of salt concentrations, from 10 to 50 g l^{-1} NaCl (Kirkwood et al. 2008).

15.3.4 Salterns

Salterns are man-made shallow ponds used for the production of salt from seawater. Most saltern systems are located in tropical and subtropical coastal areas. After evaporation in the first set of ponds has caused a sufficient rise in salinity, the water is transferred to the next set of ponds, and so on, until NaCl-saturated brine saturated is obtained from which halite precipitates in the final set of crystallizer ponds. The salinity in each pond is kept approximately constant, and planktonic and benthic microbial communities develop in each ponds, adapted to the prevailing salinity. Before halite crystallizes, other salts, notably gypsum ($CaSO_4.2H_2O$) precipitate, and thick gypsum deposits are characteristically found on the bottom of part of the saltern. Descriptions of the arrangement and operation of solar salterns were given by Davis (1978) and Javor (1989, 2002).

Benthic cyanobacterial mats are a conspicuous part of the biota of saltern ponds, from the first seawater evaporation ponds up to salinity of about 200 g l^{-1}. The cyanobacterial mats enhance solar radiation absorption, and this increases evaporation rates. The cyanobacterial mats also prevent seepage loss through the bottom sediments. In view of the valuable contribution that the cyanobacterial mats play in the operation of the salt production process, inventories of cyanobacterial species have been made in solar salt facilities all over the world. The types of cyanobacteria found at the different salinities, and the vertical arrangement of these cyanobacteria in the stratified sediments on the bottom of the ponds are similar in saltern facilities worldwide (Bauld 1981; Davis and Giordano 1996; Oren 2002, 2005, 2009a). When comparing the descriptions below, it should be realized that different names are sometimes used by different authors for the same organism (see Sect. 15.2).

The saltern systems most extensively studied for their cyanobacteria are those of Guerrero Negro (Baja California), Salins-de-Giraud on the Mediterranean coast of France, and Eilat on the Red Sea coast of Israel. Most of the discussions below of the benthic mats in the evaporation ponds up to 100–120 g l^{-1} salt and subsequently in the gypsum crusts that precipitate between 150 and 200 g l^{-1} refer to these salterns.

Filamentous cyanobacteria dominate in the extensive saltflats of Baja California, Mexico, which serve as the first stage of seawater evaporation in the production of solar salt by the Exportadora de Sal, S.A. (Javor 1989). In the Guerrero Negro salt flats the main component of the cyanobacterial community is the filamentous *Microcoleus chthonoplastes* that occurs in bundles, and forming coherent, highly productive mats at salinities between 60 and 125 g l^{-1}. *Microcoleus* is accompanied by *Oscillatoria* sp. *Microcoleus* is less abundant at salt concentrations exceeding 150 g l^{-1}. At higher salinities species belonging to the genera *Phormidium, Spirulina, Aphanothece* and *Synechococcus* predominate (Des Marais 1995; Javor 1989). The molecular diversity of cyanobacteria along the salinity gradient in the evaporation ponds of Guerrero Negro was studied based on characterization of 16S rRNA genes. Up to 110 g l^{-1} salt *Microcoleus* dominated (Canfield and Des Marais 1993; D'Antoni D'Amelio et al. 1987, 1989), while at 140 g l^{-1} *Aphanothece* and *Halospirulina* were abundantly found. A sequence related to *Oscillatoria limnetica* (*Phormidium hypolimneticum*) was also detected at 140 g l^{-1} salt. Below 60 g l^{-1} salt eukaryotic algae and higher plants cyanobacteria were outcompeted by higher plants (Nübel et al. 2000a), but no great changes in species composition (*Microcoleus*, *Oscillatoria*-like) was observed when the mats were equilibrated at a lowered salinity (35 g l^{-1}) (Green et al. 2008). The number of phylogenetic types of cyanobacteria recognized in the mats (60–210 g l^{-1}) was more than twice the number of morphotypes observed microscopically, suggesting that some morphotypes may represent different genotypes (Nübel et al. 1999). The Guerrero Negro *Microcoleus* mats were used by NASA in a long-term greenhouse experiment, simulating Earth's present and past field environments, while monitoring photosynthesis and respiration with microelectrodes and measuring other parameters of the communities. A salinity increase from 90 to 120 g l^{-1} resulted in more orange colored mats due to an increase of carotenoid content of the *Microcoleus* cells as response to increased oxidative stress. The number of *Aphanothece*-type cells also increased with salinity (Bebout et al. 2002). One *Synechococcus* isolate from the Baja California saltflats was sent into space, and survived a 2-week exposure to high solar UV radiation and vacuum in the space environment (Mancinelli et al. 1998).

Confocal laser microscopy and molecular approaches were used to characterize the cyanobacterial communities in the 72–94 g l^{-1} salt evaporation ponds of Salins-de-Giraud. *Microcoleus* was the dominant type, with a minor contribution of *Halomicronema*. Both were distributed homogeneously during the night and concentrated in the upper 1.5 mm oxic zone during the day. Additional types detected were a yet unidentified picocyanobacterium forming filaments of about 0.96 mm diameter, *Pseudanabaena* sp. and unicellular species of the *Gloeocapsa* and *Pleurocapsa* group (Fourçans et al. 2006). Caumette et al. (1994) reported occurrence of *Phormidium* (*Lyngbya*). Data on the lipid composition of these mats are also available (Rontani and Volkman 2005). Manipulation of the salinity and temperature of the Salins-de-Giraud *Microcoleus* mats showed that salinity had the highest impact on photosynthesis rates; these dropped sharply above the in situ salinity of around 100 g l^{-1} (Wieland and Kühl 2006). When *Microcoleus* mats were transplanted from 65 g l^{-1} to a lowered salinity (30–60 g l^{-1}), herbivorous snails fed on the cyanobacteria and green macroalgae (*Cladophora, Enteromorpha*) outcompeted the cyanobacteria (Cornée et al. 1992).

Other sites in which the cyanobacterial community in saltern evaporation ponds was studied include:

- La Trinitat saltern, Ebro Delta, Spain. Here *Phormidium valderianum* and *Microcoleus chthonoplastes* were reported as the predominant primary producers in the sediments at 70–100 g l^{-1} salt, with minor contributions of *Gloeocapa* and *Aphanothece*. The dominant fatty acids in the *Phormidium* mat were n-16:0, n-16:1 Δ^9 and n-18:1 Δ^9; n-18:2 was abundant in *Microcoleus* mats, but less so in *Phormidium* (Grimalt et al. 1992).
- The salterns of Cabo Rojo, Puerto Rico. Here the mats contained *Microcoleus, Johannesbaptistia,* and *Gloeocapsa*. Photosynthetic activity in the mats was much higher in the wet season (40–150 g l^{-1} salinity) than in the dry period (150–265 g l^{-1}) (Casillas-Martinez et al. 2005).
- In a saltern in the Bretagne, France, and in a laboratory model set up to simulate biological processes in the saltern ecosystem, *Aphanothece, Aphanocapsa* (*Synechocystis*)*, Microcoleus, Spirulina* and *Oscillatoria* were found (Giani et al. 1989).
- During a survey of the cyanobacteria in the benthic mats of a saltern in Egypt, *Chroococcus, Aphanothece, Aphanocapsa, Microcoleus, Oscillatoria, Spirulina* and *Phormidium* were observed, with a clear stratification: a top 2–5 mm thick layer dominated by *Aphanothece, Aphanocapsa* and *Chroococcus,* followed by a light green layer (0.2–0.9 mm thick) with *Aphanocapsa, Aphanothece, Microcoleus, Spirulina* and *Oscillatoria*, and a dark green layer (0.5–1 mm) with *Spirulina, Oscillatoria* and *Phormidium* (Taher et al. 1995).
- A checklist of species found in an Indian saltern system included *Anacystis dimidiatus, Coccochloris elabens, Gloeocapsa* sp., *Lyngbya majuscula, Oscillatoria salina, Oscillatoria formosa, Spirulina platensis*, and *Xenococcus acervatus,* all tolerating salinities of up to 174–188 g l^{-1} (Rahaman et al. 1993). Sixty-one species of cyanobacteria were identified in saltpans at the southeastern coast of India at salinities between 48 and 185 g l^{-1}. *Spirulina, Oscillatoria, Phormidium*, and *Microcoleus* were most abundant, *Aphanothece, Arthrospira, Dermocarpa* and *Synechocystis* being restricted to the lower salinities (Nagasathya and Thajuddin 2008).
- Little attention has been devoted yet to the occurrence of planktonic cyanobacteria in saltern evaporation ponds. A study of the phototrophic communities in the brines of the Santa Pola salterns, Alicante, Spain listed *Aphanothece, Spirulina*, and non-identified morphotypes of Chroococcales and Oscillatoriales (Estrada et al. 2004). Planktonic *Dactylococcopsis* and/or *Synechococcus* were reported in salterns of the Bahamas, Jamaica, Israel, Mexico and South America (Davis and Giordano 1996; Golubic 1980).

Gypsum crusts occurring in saltern ponds of intermediate salinities are generally densely colonized by different types of cyanobacteria, occurring in distinct layers. The biota of the gypsum crusts developing in the Salins-de-Giraud salterns on the Mediterranean French coast in the Rhone delta were well characterized (Caumette et al. 1994). Gypsum crusts were found at salt concentrations between 130 and 200 g l^{-1}. On top of the gypsum crystal layer, a 1–2 mm thick layer of *Aphanothece* (*Cyanothece*) was present, embedded in mucoid substance, and imparted a yellow-brown color to the bottom of the ponds during the summer months. Below the 2 mm thick layer of parallel oriented gypsum crystals, an approximately 1–2 mm thick green layer was found, inhabited by filamentous cyanobacteria of the genus *Phormidium*. Similar systems have been reported from salterns in Spain (Ortí Cabo et al. 1984; Thomas 1984), Australia (Coleman and White 1993; Jones et al. 1981; Sammy 1983), Egypt (Taher et al. 1995), Brazil (De Medeiros Rocha and Camara 1993), and India (Rahaman et al. 1993).

The properties of the biota, including the cyanobacteria, of the gypsum crusts in the salterns of Eilat, Israel, have been the subject of many studies. Yellow-orange unicellular (*Aphanothece* and *Synechococcus*-type cells) occur in the upper 1–2 cm, and a 2–4 mm thick green layer of *Phormidium* filaments is present below (Fig. 15.2). Below the cyanobacterial layers, a purple layer of anoxygenic phototrophic bacteria is found. Aspects studied here include:

- Pigment content and light penetration to the different layers of phototrophs. The brown color of the upper layer was due to the high carotenoid content of the cyanobacteria (mainly echinenone and myxoxanthophyll). Use of optic fiber microprobes showed that about 1% of the photosynthetically active radiation reached the green layer (Oren et al. 1995).
- The types of photosynthetic pigments present in the different layers, using emission spectroscopy and kinetic fluorimetry techniques (Prášil et al. 2009)
- The presence of UV-absorbing pigments. The upper orange layer populated by *Aphanothece*-type unicellular cyanobacteria possessed an extremely high absorption in the near UV range, with a peak around 332 nm and a shoulder around 365 nm. This absorption is due to an extremely high content of two mycosporine-like amino acids (MAA's) (Oren 1997). Such UV-absorbing pigments are probably also present in similar gypsum crusts elsewhere: spectra of the upper cyanobacterial layer in the gypsum crust of the Salins-de-Giraud saltern showed a sharp rise in absorbance from 390 to 350 nm (the lowest wavelength measured) (Caumette et al. 1994). The two MAA's found in the unicellular cyanobacteria from Eilat have been identified (Kedar et al. 2002; Volkmann et al. 2006) (see also Sect. 15.6.2.4).

Fig. 15.2 A gypsum crust from an evaporation pond (approximately 200 g l^{-1} salt) of the salterns in Eilat, Israel, showing *coloured layers* of unicellular cyanobacteria (*Aphanothece – Euhalothece* type) (*orange*), filamentous cyanobacteria (*Phormidium* type) and photosynthetic sulphur bacteria (*purple*) (Photograph: Dr Rhena Schumann, University of Rostock)

- Photosynthetic activity measurements using oxygen microelectrodes (Canfield et al. 2004; Sørensen et al. 2009) and planar optodes in incubation chambers (Woelfel et al. 2009). Due to the relatively low rates of oxygen production and the deep photic zones extending from 1.5 to 3 cm depth, a large percentage of the oxygen produced accumulated within the crust, and only 16–34% of the oxygen produced escaped (Canfield et al. 2004). Microelectrode studies showed that oxygen formation peaked at 198 g l^{-1} and decreased only slightly at lower salinities. Slurry experiments indicated a broad range of optimum salinity for CO_2 photoassimilation between 80 and 230 g l^{-1} (Oren et al. 2009; Sørensen et al. 2004).
- The occurrence of photosystem II-independent anoxygenic photosynthesis in the *Phormidium* layer, which is exposed to sulphide and anaerobic conditions much of the time (Canfield et al. 2004). Light-dependent CO_2 photoassimilation could be demonstrated in this layer in the presence of DCMU and 1–2 mM sulphide (Ionescu et al. 2007) (Sect. 15.5).
- The distribution of fatty acids in the orange and green cyanobacterial communties, with special emphasis on the possible mode of biosynthesis of unsaturated fatty acids. The upper orange *Aphanothece* layer had a high content of di-unsaturated fatty acids (21% and 7% 16:2 *cis* Δ^7, Δ^{10} and 18:2 *cis* Δ^9, Δ^{12}, respectively). No polyunsaturated fatty acids, whose biosynthesis is always oxygen-dependent, were found in the green layer. There the dominant mono-unsaturated fatty acids were 16:1 *cis* Δ^7 (16.3 mol%) (and not 16:1 *cis* Δ^9 as in the upper orange layer) and 18:1 *cis* Δ^9 (27.9 mol%). The nature of the fatty acids in the green layer suggests that the cyanobacteria use an oxygen-independent pathway for production of unsaturated fatty acids, a pathway rarely encountered in cyanobacteria (Oren et al. 2009; Ionescu et al. 2007).

The benthic cyanobacterial mats contribute greatly to the salt production process in solar saltern ponds. Increased solar absorption caused by the pigmented microorganisms leads to elevated temperatures, which help to offset the effects of the low vapour pressures, and thus permit adequate evaporation. In addition, the mats strip and sequester minerals from the overlying brine and control brine leakage (Davis 1974, 1978, 1993; Javor 2002; Jones et al. 1981). Excessive development of cyanobacteria, however, is not desirable as their extracellular polysaccharides interfere with the production of good quality salt in the crystallizer ponds. Therefore different management procedures have been proposed to promote optimal development of the mats in the evaporation ponds while minimizing release of polysaccharide slime to the brine (Coleman and White 1993; Davis 1993; De Medeiros Rocha and Camara 1993; Rahaman et al. 1993; Sammy 1983).

15.4 Physiological Properties of the Major Halophilic Cyanobacteria

Although a wide range of cyanobacteria, belonging to different taxonomic groups, were reported to live at high salinities, only a few taxa have been studied in-depth. These include the unicellular *Aphanothece halophytica* (including isolates designated *Euhalothece, Halothece*, etc., see Sect. 15.2), the cosmopolitan mat-building filamentous *Microcoleus chthonoplastes* found from seawater salinity to salinities exceeding 200 g l^{-1}, and the recently characterized species *Halomicronema excentricum* and *Halospirulina tapeticola*.

15.4.1 *Aphanothece halophytica – Euhalothece – Halothece* Group

Aphanothece halophytica, found worldwide in hypersaline environments, was first described by Hof and Frémy (1933). It has been extensively studied since Brock (1976) recognized its importance as a model organism for the study of phototrophic life at high salt concentrations.

Significant differences in shapes and size were noted for this form, and cell morphology variations complicate the taxonomic picture. The morphological variability was already mentioned by Hof and Frémy (1933). Yopp et al. (1978a) described their pure culture as consisting of cells

variable in size (between 2 and 10 μm) and shape (ellipsoidal, ovoid or cylindrical). Cell size increases with salinity (Berland et al. 1989; Dor and Hornoff 1985; Kao et al. 1973; Yopp et al. 1978a). Morphology mutants have also been isolated (Yopp et al. 1979).

16S rRNA sequence-based phylogenetic studies have in recent years led to a reassessment of the taxonomy of the group of *Aphanothece* and similar organisms (Garcia-Pichel et al. 1998; Margheri et al. 1999, 2008). Based on a comparative analysis of a large number of isolates, the name *Euhalothece* was proposed for a cluster of strains (Garcia-Pichel et al. 1998), and another deep-branching strain was named *Halothece californiensis* (Margheri et al. 2008). In most cases it is impossible to ascertain to which of these taxa the strains used in the experimental studies described below may have belonged. For the sake of convenience the name *A. halophytica* is used in the examples cited.

A. halophytica is mostly found as a benthic species. Its populations cover light-exposed surfaces of sediments and evaporite crusts. Examples of such benthic development of *Aphanothece* can be found in Great Salt Lake, Utah (Brock 1976; Post 1977) and in saltern ponds (Caumette et al. 1994; Margheri et al. 1987; Oren et al. 1995). In Solar Lake, Sinai, *A. halophytica* was found as a planktonic species, and it formed the dominant component of the phytoplankton during the summer season when the lake was mixed and salinity was high (around 180 $g\,l^{-1}$) (Cohen et al. 1977b). A planktonic strain isolated from Solar Lake was used in studies of the cell wall proteins and their possible relation to cell motility (Simon 1981), anoxygenic photosynthesis (Garlick et al. 1977), the properties of RUBISCO (Asami et al. 1983), and the biosorption of zinc ions (Incharoensakdi and Kitjaharn 2002).

A. halophytica requires a minimum salt concentration of around 30 $g\,l^{-1}$ and grows up to salt concentrations as high as 300–350 $g\,l^{-1}$. Most studies indicated optima in the range of 60–150 $g\,l^{-1}$ (Borowitzka 1981; Brock 1976; Kao et al. 1973; Tindall et al. 1978; Yopp et al. 1978a). Also CO_2 fixation rates were much higher in the presence of 2 M than at 0.25 M NaCl (Takabe et al. 1988). The species displayed a specific requirement for sodium ions, which could not be replaced by potassium or by lithium; glycerol did not substitute for salt (Kao et al. 1973). Doubling times of 14.5, 18 and 30 h were measured in the presence of 2, 3, and 4 M NaCl, with an optimal pH of 7–7.8 (minimum pH for growth 6.4) (Tindall et al. 1978).

A. halophytica uses glycine betaine as osmotic solute (Sect. 15.6.2.3). When exposed to a sudden increase in NaCl concentration from 0.25 to 1 or 2 M, growth temporarily ceased, to resume after 0.5–2 days. After the adaptation period the growth rate was similar to that at the low salt concentration before, but the capacity of the cells for CO_2 fixation when adapted to 2 M NaCl was 3.7 times higher than of cells growing at 0.25 M NaCl. High-NaCl-grown cells also contained increased levels of RUBISCO. The latter perhaps was required for the synthesis of the large amounts of osmotic solute needed for growth at high salt concentrations (Takabe et al. 1988).

The fact that *A. halophytica* maintains its osmotic balance by accumulation of organic osmotic solutes and keeps intracellular ionic concentrations low is also evident from the fact that its RUBISCO was inhibited by low concentrations (more than 50% inhibition at 0.25 M) of chloride (but not by sodium of potassium ions; Incharoensakdi and Takabe 1988). Low concentrations of sodium ions (0.25 M) promoted the dissociation of the small subunits from the molecule (Asami et al. 1983). As in vivo sodium concentrations are expected to be low, the importance of this phenomenon in vivo is not clear. A molecular chaperonin encoded by the gene *dnaK* has been characterized. Its genomic transcript increased after heat stress and also upon transfer to a hyperosmotic environment, suggesting a role of DnaK in the recovery of *A. halophytica* following hyperosmotic stress (Lee et al. 1997; see Sect. 15.7 for potential biotechnological applications of this chaperonin).

Other cellular components of *A. halophytica* characterized included C-phycocyanin (Kao et al. 1973) and the plasma membrane lipids. Monogalactosyldiacylglycerol was the most abundant lipid in the plasma membrane of cells grown at 1 M NaCl, while in cells adapted to 3 M NaCl phosphatidylglycerol dominated. The decreasing ratio of uncharged to charged lipids with increasing external salinity was claimed to increase membrane stability in the presence of high salt and to enable specific modulation of membrane-associated enzyme activity (Ritter and Yopp 1993).

A. halophytica communities are often associated with copious amounts of slime (Javor 1989; Oren et al. 1995). Three independent analyses of the chemical composition of the slime formed by *A. halophytica* gave greatly different results. This may be due to the use of different isolates. The "*Cyanothece*" strain isolated from Somalia had a polysaccharide capsule, the external part of which dissolves in the medium during growth, causing an increase in medium viscosity. Glucose and mannose to be the most abundant sugars, glucose, mannose, xylose, galactose, galacturonic acid, fucose, and glucuronic acid occurring in the ratio of 6.8:4.8:2.9:2.4:2:1.6:1 (De Philippis et al. 1993). Arabinose, ribose, and rhamnose were also detected in the polysaccharides of certain *Cyanothece* strains, and acetyl, pyruvyl and/or sulphate groups may also be present (De Philippis et al. 1998). Uronic acids were absent from the exopolysaccharide formed by an *A. halophytica* strain isolated from an unspecified salt lake, and here fucose was the most abundant sugar (fucose, glucose, mannose, galactose, xylose and rhamnose being found in the ratio 53:25:15:3:3:2, respectively). Proteins were also present in the slime to about 10%

of the total weight, and many of the sugar residues carried sulphate groups (up to 12% by weight). Polysaccharide production increased with increasing nitrate concentration of the medium, and was optimal in the low salinity range (20–50 g l^{-1} NaCl) (Sudo et al. 1995). In the strain investigated by Jones and Yopp (1979), polysaccharide excretion was similar over the whole range of salt concentrations, but was greatly increased when the cultures reached the stationary growth phase. A similar result was obtained with an isolate designated *Synechococcus* from a gypsum crust from a saltern in Western Australia. Nutrient limitation activated extracellular polysaccharide production (Roux 1996). Analysis of the slime showed presence of glucose, fucose, mannose, and galactose in the ratio 1:0.6:0.32:0.23, with 5% minor constituents. Uronic acids or ninhydrin-positive compounds were not detected. An analysis of the polysaccharide slime of *A. halophytica* strain GR02 yielded a major fraction (apparent molecular mass 2×10^6 Da) containing glucose, fucose, mannose, arabinose, and glucoronic acid, and a minor fraction with rhamnose, mannose, fucose, glucose, galactose and glucuronic acid and traces of arabinose (Li et al. 2001). The presence of copious amounts of polysaccharides surrounding the cells makes isolation of *A. halophytica* in pure culture difficult. Thus, the axenic culture of a strain from a Californian solar evaporation pond was achieved through a protocol involving treatment with different antibiotics, density gradient centrifugation, and ultraviolet radiation (Yopp et al. 1978a).

A. halophytica can fix nitrogen and has the capacity of anoxygenic photosynthesis with sulphide as electron donor. Nitrogen fixation (acetylene reduction) was detected in evaporite crusts from Guerrero Negro, Baja California, and was attributed to the presence of a "*Synechococcus*", being probably identical to *A. halophytica*. The evaporite crust fixed N_2 in the light both aerobically and anaerobically (up to 2.4 nmol C_2H_4 produced g^{-1} evaporite h^{-1}), but no dark fixation was found (Rothschild et al. 1994). Sulphide-dependent anoxygenic photosynthesis was detected in the Solar Lake strain of *A. halophytica* (Garlick et al. 1977).

15.4.2 *Microcoleus (Coleofasciculus) chthonoplastes*

Microcoleus chthonoplastes is found in a wide range of ecosystems of greatly differing salinities, from marine (Chap. 4) to hypersaline, at salt concentrations exceeding 200 g l^{-1}, and even up to 300 g l^{-1} (Zavarzin et al. 1993; Schneider and Herrmann 1980) (Fig.15.1). To separate the halophilic *M. chthonoplastes* from phylogenetically unrelated, non-halophilic species classified in the genus *Microcoleus*, the halophilic forms were recently reclassified as *Coleofasciculus chthonoplastes* (Thur. ex Gomont) Siegesmund *et al.* comb. nov. (Siegesmund et al. 2008). For the sake of convenience the older name is used here.

M. chthonoplastes strains isolated from habitats all over the world appeared phenotypically and phylogenetically indistinguishable (Garcia-Pichel et al. 1996). However, physiological ecotypes may be distinguished that differ in their optimal salinity for growth and their maximum growth rates, as related to the habitat from which the strains were isolated (Karsten 1996). A comparative chemosystematic study of the carotenoids and mycosporine-like amino acid compounds in members of the genus *Microcoleus* has also been performed (Karsten and Garcia-Pichel 1996). Phylogenetic analysis on the basis of 16S rRNA sequences showed *Microcoleus* to be closely related to the *Oscillatoria* group (Garcia-Pichel et al. 1996).

The optimal temperature for growth of *Microcoleus* was around 30°C, and optimal photosynthesis rates were achieved at pH values around 7.5 (best between 7 and 8.5) (Dubinin et al. 1992a). However, the organism was also found in microbial mats in the highly alkaline (pH 9.5; 46.6 g l^{-1} salt) Lake Khilganta (Buryatiya) (Gerasimenko et al. 2003). Photosynthetic activity was even detected at salinities as high as 260 g l^{-1} (Dubinin et al. 1992a). An important part of the photosynthetically fixed carbon was probably used for the synthesis of glucosylglycerol, the organic osmotic solute accumulated by *Microcoleus*, used to achieve osmotic balance with the high salt concentrations of the surrounding medium (Sect. 15.6.2.2).

Migration of *Microcoleus* filaments may be driven by salinity in a process termed 'halotaxis' (Kohls et al. 2010). In an intertidal hypersaline cyanobacterial mat from Abu Dhabi, *Microcoleus* was found to migrate up and down when salinity was decreased below or increased above 15%, respectively, causing a colour change of the mat uppermost layer. Migration was independent of light and other physico-chemical parameters such as water activity, oxygen solubility, and sulfide, indicating that the observed migration was due to a direct response to salt stress.

Microcoleus filaments are often found at or near the interface between the aerobic upper layer of the sediment and the anaerobic deeper layers where dissimilatory sulphate reduction occurs. Filaments often are exposed to sulphide, especially at the end of the night and in early morning, when sulphide produced in the sediment diffuses upward, and sulphide utilization by photosynthetic sulphur bacteria is limited because of lack of available light. Though not as versatile as *Oscillatoria limnetica* from Solar Lake, which is able to grow with high concentrations of sulphide as electron donor (Oren and Padan 1978; Oren et al. 1985), *Microcoleus* can grow in the presence of 0.15 mM sulphide, and low concentrations of sulphide can actually enhance oxygenic photosynthesis rates (Cohen 1984b); at concentrations exceeding 1 mM growth is inhibited (de Wit et al. 1988). Sulphide was used as electron

donor in an anoxygenic type of photosynthesis, in which thiosulphate was the end product of sulphide oxidation (de Wit et al. 1988). Field studies with microelectrodes suggest that oxygenic and anoxygenic photosynthesis occurred simultaneously in *Microcoleus* mats (Jørgensen et al. 1986). *Microcoleus*, like most other filamentous cyanobacteria, contains polyunsaturated fatty acids (14:2, 18:2 and 18:3), the biosynthesis of which is oxygen-dependent. As a consequence, no real anaerobic growth will occur. However, in the presence of oxygen sulphide can drive growth also without the participation of photosystem II (de Wit et al. 1988).

As an adaptation to prolonged exposure to anaerobic conditions, *M. chthonoplastes* also possesses a well developed fermentative metabolism that permits fermentation of endogenous carbohydrate reserves, with acetate as the main product (Moezelaar et al. 1996). Interesting interactions occur between the cyanobacterium *Microcoleus* and halophilic Archaea in the microbial mats of the Sivash lagoon (Arabat, Crimea) at salinities between 150 and 300 $g\,l^{-1}$. In these mats, at least 51% of the aerobic heterotrophic bacteria recovered as colonies were halophilic Archaea, and this percentage increased to 70–80% at the higher salinities examined. The genera most frequently isolated were *Haloarcula* and *Halorubrum*. These Archaea were thus considered as ecologically significant components of the cyanobacterial mat community. Cultivation experiments of *Microcoleus* together with halophilic Archaea at 150 $g\,l^{-1}$ showed the existence of interesting metabolic interactions. *Microcoleus* secreted a number of organic acids into the medium and these were used as carbon sources for the Archaea. Paper chromatography of the medium components after incubation of *Microcoleus* in the presence of $^{14}CO_2$ showed the presence of acetate, butyrate, α-ketoglutarate, succinate, malate, fumarate and citrate (Zvyagintseva et al. 1995).

Microcoleus also can use elemental sulphur as an electron acceptor for anaerobic respiration and this leads to the formation of H_2S in the dark (Dubinin and Gerasimenko 1993; Moezelaar et al. 1996). An interesting association of the aerobic *M. chthonoplastes* with presumably anaerobic filamentous purple bacteria may exist within *Microcoleus* bundles. Electron microscopic examination of microbial mats of Solar Lake (82 $g\,l^{-1}$ salinity) and Guerrero Negro (72–91 $g\,l^{-1}$) showed the occurrence of gliding filamentous phototrophic purple bacteria with stacked photosynthetic lamellae between the *Microcoleus* filaments within the common sheath (D'Antoni D'Amelio et al. 1987, 1989). One possibility is that these purple bacteria use toxic sulphide to which the cyanobacterial bundles were often exposed while living in sharp gradients of oxygen and sulphide. Alternatively, they may live on elemental sulphur and thiosulphate formed by *Microcoleus* in the process of anoxygenic photosynthesis (Sect. 15.5), or grow photoheterotrophically on organic compounds excreted by the cyanobacterium. The filamentous purple bacterium was not cultured, and a further analysis of the interactions between the halophilic cyanobacterium and the anoxygenic phototrophic bacterium remains to be completed.

Microcoleus accumulates peroxides during oxygenic photosynthesis, and under certain conditions up to 40% of the electron flow through the electron transport chain may be used for peroxide formation (Dubinin et al. 1992b). As *Microcoleus* lacks catalase, peroxide accumulation can easily lead to growth inhibition. The cyanobacterium therefore may rely on the catalase activity in the heterotrophic components of the microbial mat community to prevent autoinhibition of *Microcoleus* by peroxide (Dubinin et al. 1992b).

Although nitrogen fixation was sometimes observed in microbial mats dominated by *Microcoleus* (de Wit et al. 2005; Steppe et al. 1996), there is no evidence that the organism contains nitrogenase genes.

15.4.3 *Halospirulina tapeticola*

Tightly coiled cyanobacterial filaments have been observed in hypersaline microbial mats in many locations worldwide at salinities around 100 $g\,l^{-1}$ (e.g. D'Antoni D'Amelio et al. 1989; Canfield and Des Marais 1993). A comparative study of isolates of cyanobacteria with helical tightly-coiled trichomes (in the past assigned to the genus *Spirulina*) showed large genetic divergence. A monophyletic cluster of highly halotolerant, euryhaline organisms with elevated temperature tolerance and showing gliding motility was reclassified as *Halospirulina tapeticola*. The isolates grow from 1.6–3.2 to up to 16–20 $g\,l^{-1}$ salt (Nübel et al. 2000b).

15.4.4 *Halomicronema excentricum*

Halomicronema excentricum is a recently described halophilic non-heterocystous filamentous cyanobacterium (Abed et al. 2002). Its filaments consist of cells less than 2 μm in diameter and slightly less than 1 μm wide. Groups of a few gas vesicles are typically present near the cross walls. A number of isolates were obtained from a microbial mat derived from Solar Lake, Sinai. The organism grows optimally at temperatures from 28°C to 50°C and salinities between 32 and 120 $g\,l^{-1}$, and tolerates up to 150 $g\,l^{-1}$ salt.

15.5 Anoxygenic Photosynthesis by Cyanobacteria in Hypersaline Environments

In many hypersaline environments cyanobacterial populations are exposed to high concentrations of sulphide, either permanently or periodically. The sulphide may be of geothermal

origin, as is the case in certain sulphur springs, but in most cases it is derived from dissimilatory sulphate reduction, which is the most important terminal process in anaerobic degradation in marine environments.

Certain species of cyanobacteria can use sulphide as electron donor in an anoxygenic type of photosynthesis, producing elemental sulphur or thiosulphate and using photosystem I only (Padan 1979a, b). No direct link exists between halophilism and anoxygenic photosynthesis, and sulphide-dependent anoxygenic photosynthesis was also detected in some freshwater environments (Cohen 1984b; Padan 1979b). However, the most comprehensive studies on anoxygenic photosynthesis in cyanobacteria were performed in the hypersaline Solar Lake. The halophilic Solar Lake isolate *Oscillatoria limnetica* became a laboratory model for the study of the biochemistry of anoxygenic photosynthesis in cyanobacteria.

As described above (Sect. 15.3.1.3), Solar Lake is stratified in winter. The hypolimnion, below a depth of 1–2 m, is anaerobic, and becomes rich in sulphide (1 mM and higher) as a result of high rates of dissimilatory sulphate reduction in the bottom sediments. The temperature of the hypolimnion brines (salinity around 180 $g l^{-1}$) rises during winter stratification to values as high as 61°C due to heliothermal heating (Cohen et al. 1975a, 1977a). The high transparency of the shallow upper layer allows good light penetration down into the hypolimnion, so that dense communities of sulphide-utilizing anoxygenic phototrophs can develop there (Cohen et al. 1977b).

The filamentous species identified as *Oscillatoria limnetica* (see Sect. 15.2) forms dense blooms at the lower end of the hypolimnion near the bottom, where sulphide concentrations are highest. This organism can shift between two types of photosynthesis: oxygenic photosynthesis in which two photosystems cooperate to split water and allow CO_2 photoassimilation, and anoxygenic photosynthesis in which sulphide serves as electron donor in a process which does not require participation of photosystem II. Accordingly, DCMU, an inhibitor of photosynthetic electron transport at the acceptor side of photosystem II, does not inhibit sulphide-dependent CO_2 photoassimilation (Cohen et al. 1975b). Photosystem II does not participate in the process, as shown from a comparison of photosynthetic action spectra in the presence and absence of DCMU (Oren et al. 1977). The phenomenon is due to the fact that low concentrations of sulphide inhibit electron flow at the donor side of photosystem II (Oren et al. 1979). It other systems examined, the participation of photosystem II to the photosynthetic processes in the presence of sulphide was significant (Cohen 1984b). The optimal sulphide concentration for the process was around 2–3 mM, and elemental sulphur was the sole product of sulphide oxidation (Cohen et al. 1975c). The shift from oxygenic to anoxygenic photosynthesis required a lag period of a few hours (Oren and Padan 1978; Shahak et al. 1987). Induction involves synthesis of a sulphide-quinone reductase, the key enzyme required to feed electrons from sulphide into the photosynthetic electron transport chain between the acceptor site of photosystem II and the donor site of photosystem I (Arieli et al. 1991). This enzyme was characterized and heterologously expressed in *Escherichia coli* (Arieli et al. 1994; Bronstein et al. 2000). In addition, a 11.5 kDa periplasmic protein is induced that may play a role in transport of sulphide into the cells or extrusion of elemental sulphur (Arieli et al. 1989).

O. limnetica can fix CO_2 in the light in the presence of high sulphide concentrations, and grows anaerobically in the presence of sulphide (Oren and Padan 1978). Growth in the absence of molecular oxygen requires special adaptations such as the absence of polyunsaturated fatty acids (abundant in the membrane lipids of most filamentous cyanobacteria) and the presence of an oxygen-independent pathway to synthesize monounsaturated fatty acids. *O. limnetica* lacks polyenoic acids (Oren et al. 1985), and produces monoenoic acids also under anaerobic conditions: upon anaerobic incubation in the presence of sulphide and DCMU, the types of monoenoic fatty acids formed agreed with the functioning of the bacterial, anaerobic pathway of synthesis of unsaturated fatty acids rather than by the oxygen-requiring desaturation of the saturated equivalents 16:0 and 18:0 (Jahnke et al. 1989).

Another adaptation of *O. limnetica* to anaerobic life is the presence of different modes of energy generation under anaerobic conditions in the dark. One such mechanism involves anaerobic respiration of endogenous storage materials, using elemental sulphur (formed during daytime by photosynthetic oxidation of sulphide) as electron acceptor, with the formation of CO_2 and sulphide (Oren and Shilo 1979). Indications exist that this type of metabolism may be operative in Solar Lake, where sulphide formation at the expense of elemental sulphur was observed below the chemocline during the night (Jørgensen et al. 1979). Another mode of anaerobic life that reflects the metabolic flexibility of *O. limnetica* is fermentation of endogenous carbon reserves, with the formation of lactate as the main product (Oren and Shilo 1979).

An *Aphanothece halophytica* isolate from Solar Lake also had a capacity to use sulphide as electron donor in anoxygenic photosynthesis (Garlick et al. 1977; Shabana and Ali 1999). However, this form was much less sulphide tolerant than *O. limnetica*, and the optimal sulphide concentration for the process was 0.7 mM only. Also in the *Aphanothece* strain, elemental sulphur was identified as the product of sulphide oxidation (Garlick et al. 1977). *Microcoleus* in the Solar Lake microbial mat is another versatile organism that may use oxygenic and anoxygenic photosynthesis simultaneously in the presence of low sulphide concentrations. In this case

photosystem II was partially inhibited, and anoxygenic photosynthesis was partially induced (Cohen 1984b; Cohen et al. 1986; Jørgensen et al. 1986).

Anoxygenic photosynthesis with sulphide as electron donor was implicated as an ecologically important process in a number of additional hypersaline environments:

- In the lagoons of Lake Sivash (east Crimea), *Microcoleus* mats used sulphide as electron donor, which was oxidized to thiosulphate (Zavarzin et al. 1993).
- In a microbial mat in a hypersaline (74 $g l^{-1}$) lagoon on San Salvador Island, Bahamas, DCMU-amended samples had 25% of photosynthetic activity of the control. This anoxygenic photosynthetic activity was possibly due to *Microcoleus* (Pinckney and Paerl 1997).
- In the gypsum crusts deposited on the bottom of the saltern ponds in Eilat (see Sect. 15.3.4), a layer of green *Phormidium*-type filamentous cyanobacteria (Fig.15.2) is exposed most of the day and night to anaerobic conditions and presence of sulphide (Canfield et al. 2004). Photosynthetic activity (CO_2 photoassimilation) in the presence of sulphide (optimum: 1–2 mM) proceeded also in the presence of DCMU. Moreover, the organism appears to use an oxygen-independent pathway for the biosynthesis of unsaturated fatty acids, showing its high degree of adaptation toward anaerobic life (Ionescu et al. 2007).
- At the time when there still was flow of hypersaline sulphide-containing waters from the warm springs of Hamei Mazor on the shore of the Dead Sea, the filamentous *Phormidium*-like cyanobacteria that covered the bottom of the springs and the outflow channels showed DCMU-insensitive CO_2 photoassimilation in the presence of sulphide (Oren 1989).

15.6 Osmotic Adaptation of Cyanobacteria Living at High Salt Concentrations

To withstand the high osmotic pressure exerted by the salt concentrations in their surrounding medium, cyanobacteria living at high salinities possess mechanisms to maintain osmotic equilibrium and cell turgor. While ions (Na^+, K^+, Cl^-) can temporarily enter the cells to counteract rapid increases in medium salinity, in the long term, organic solutes are accumulated to provide osmotic balance, as required in organisms whose enzymatic machinery does not operate properly at high salt concentrations. Different organic osmolytes occur in cyanobacteria: the disaccharides sucrose and trehalose (especially in the less salt tolerant types); glucosylglycerol (in species with a moderately halotolerance); and glycine betaine (in *Aphanothece halophytica* and a few other types that tolerate high salt concentrations) (Oren 2006, 2007; see also Joset et al. 1996; Hagemann et al. 1998 for general overviews of the responses of cyanobacteria to salt stress). Our understanding of the mechanisms of osmoregulation in cyanobacteria increased greatly in recent years, especially thanks to the detailed molecular analysis of osmotic adaptation in the (non-halophilic) *Synechocystis* strain PCC 6803 (Hagemann 2010).

15.6.1 Ion Metabolism

Before the discovery of organic compatible solutes it was believed that intracellular salt concentrations in halophilic cyanobacteria were quite high. Miller et al. (1976) reported intracellular potassium concentrations as high as 1 M in *Aphanothece halophytica*, with low intracellular sodium concentrations. Blue dextran was used in these studies to estimate the intracellular and extracellular space in cell pellets. Intracellular potassium concentrations increased with the salt concentration of the medium, and thus potassium was claimed to be the main osmoticum in *A. halophytica* (Miller et al. 1976; Yopp et al. 1978b).

However, claims of high intracellular salt concentrations in cyanobacteria are problematic in view of the salt sensitivity of the intracellular enzymatic systems. Based on a study of the inhibitory effect of salts on RUBISCO activity in *A. halophytica* it was concluded that in order for the enzyme to be active in vivo, the intracellular chloride concentration may not exceed 150 mM (Incharoensakdi and Takabe 1988). Later studies showed lower salt concentrations inside halophilic cyanobacteria. Intracellular chloride concentrations in *A. halophytica* were found to increase from 35 to 150 mM when the medium NaCl concentration was raised from 0.5 to 2 M (Incharoensakdi and Takabe 1988). Reed et al. (1984a) detected only low internal potassium concentrations (180–250 mM) in *A. halophytica*. Also in other species of unicellular halophilic and halotolerant cyanobacteria intracellular potassium was low – between 170 and 310 mM – in cells grown in media with between 0.5 and 4 times the salt concentration of seawater (Reed et al. 1984a). The presence of a relatively large periplasmic space may influence estimates of intracellular salt concentrations. Intracellular salt concentrations were measured in *Synechocystis* PCC 6803 – a strain which can tolerate up to 1.2 M salt – using centrifugation through a layer of silicone oil, a method which does not discriminate between the cytoplasm and periplasmic space. Cells grown at 0.65 M and 1.03 M NaCl had apparent intracellular sodium concentrations of 0.22 and 0.51 M and potassium concentrations of 0.15 and 0.30 M, respectively (Hagemann et al. 1994).

Upon sudden increases in salt concentration in the outside medium, salts may transiently accumulate to achieve a rapid osmotic balance before sufficient amounts of organic osmotic solutes are produced. The latter is a slow process that can take many hours, and it requires adaptation of the photosynthetic

apparatus. A multiphasic osmotic adjustment was documented in the euryhaline *Synechocystis* PCC 6714 upon a shift from freshwater to 0.5 M NaCl: rapid entry of NaCl permitted partial recovery of the volume within 2 min; this was followed by an exchange of sodium by potassium in 20 min, and finally accumulation of the organic osmotic solutes glucosylglycerol and sucrose enabled the extrusion of potassium over a period of 24 h (Reed et al. 1985). Transient accumulation of salt was also documented in *Spirulina subsalsa*, isolated from the Bardawil lagoon in Sinai and growing between 0.25 and 2.5 M NaCl. Following an increase in medium salinity, intracellular sodium and chloride were temporarily greatly increased. Subsequently, sodium was extruded by means of a Na^+/H^+ antiporter driven by the proton gradient generated by respiration (Gabbay-Azaria and Tel-Or 1991; Gabbay-Azaria et al. 1992). Respiration rates were enhanced following salt upshock, and cytochrome oxidase was suggested to be involved in the generation of the proton gradient required for Na^+ extrusion (Gabbay-Azaria and Tel-Or 1993). During this adaptation phase, the intracellular concentration of glycine betaine, the osmotic solute found in this organism, increased to achieve a new osmotic equilibrium. Na^+/H^+ antiporter activities were also characterized in *Aphanothece halophytica*. Two NapA-type Na^+/H^+ antiporters were found, homologous to similar antiporters of plants, mammals, and NhaP of *Pseudomonas* and SynNhaP of *Synechocystis* (Waditee et al. 2001; Wutipraditkul et al. 2005). *A. halophytica* also has a Na^+-stimulated P-type ATPase in its plasma membrane; cells grown under salt stress displayed higher ATPase activity than under non-stressed conditions; saturation was achieved at 100 mM Na^+ (Wiangnon et al. 2007). Other ion pumps studied in cyanobacteria of the *Aphanothece* group are the ΔpH-driven ATP-dependent nitrate transporter (Incharoensakdi and Laloknam 2005) and the bicarbonate transporters of an alkaliphilic *Euhalothece* strain (Mikhoudyuk et al. 2008).

Influx of sodium ions upon salt upshock may have a temporary inhibitory effect on the photosynthetic system, as suggested from studies with *Agmenellum quadruplicatum*, a marine coccoid cyanobacterium growing from 0 to over 100 g l^{-1} NaCl. Upon transfer from 18.5 to 70 g l^{-1} NaCl, photosynthesis was severely depressed and recovered only after several hours, during which time excess sodium was pumped out of the cells (Batterton and van Baalen 1971).

15.6.2 Organic Osmotic Solutes

The importance of organic osmotic solutes in the adaptation of cyanobacteria to life at high salt concentrations became clear in the early 1980s as a result of the use of techniques such as HPLC and ^{13}C-NMR spectroscopy. A survey of the occurrence of osmotic solutes showed that cyanobacteria can be divided into three groups, both according to the types of solutes accumulated and to salt tolerance: freshwater; marine; and halophilic isolates. Freshwater strains accumulate sucrose and/or trehalose under salt stress. Marine strains, adapted to the presence of seawater salinities, and often tolerating much higher salt concentrations, generally use the heteroside glucosylglycerol (2-*O*-α-D-glucopyranosyl-(1→2)-glycerol) to achieve osmotic equilibrium. Finally, the most salt tolerant strains accumulate high intracellular concentrations of glycine betaine (Mackay et al. 1984; Reed et al. 1984b). Additional osmotic solutes are sometimes found, such as L-glutamate betaine (*N*-trimethyl- L -glutamate), detected in combination with sucrose and/or trehalose in halophilic *Calothrix* isolates (Mackay et al. 1984).

The massive accumulation of organic osmotic solutes that results in intracellular concentrations in the molar range has important implications for the carbon cycle in hypersaline environments where primary production is dominated by halophilic cyanobacteria. These osmotic solutes may become available to the heterotrophic communities either when leaking out of the cyanobacterial cells, as a reaction to a decrease in salinity of the environment, or upon cell death. Methylated amines are the main precursor for methane formation in hypersaline environments and glycine betaine, the main compatible solute in the most halotolerant cyanobacterial strains (and also produced by certain anoxygenic phototrophic eubacteria) is the main precursor for the formation of these methylated amines.

15.6.2.1 Sucrose and Trehalose

The disaccharides sucrose and trehalose are of little importance for the osmotic adaptation of cyanobacteria that live at high salinities because they are not very effective as osmotic solutes, and they provide osmotic protection only up to relatively low salt concentrations. Freshwater cyanobacteria often produce sucrose and/or trehalose as a reaction to salt stress (Blumwald and Tel-Or 1982; Gabbay-Azaria and Tel-Or 1993), and even then the concentrations accumulated intracellularly may be insufficient to balance the osmotic pressure of the outside medium, as found in the case of sucrose in *Synechococcus* PCC 6311 (Blumwald et al. 1983). When grown at low salt concentrations, also *Microcoleus chthonoplastes* produces trehalose as osmotic solute, which is replaced by glucosylglycerol at higher salinities (Karsten 1996).

15.6.2.2 Glucosylglycerol

Glucosylglycerol was first identified by means of natural abundance ^{13}C-NMR in a *Synechococcus* sp. isolated from intertidal rocks in Australia, growing from 30 mM to 1.69 M total salts (Borowitzka et al. 1980). Glusosylglycerol was detected in a wide variety of salt-tolerant cyanobacteria. Some of the most widespread halotolerant species accumulate the solute, such as *Microcoleus* (Kevbrin et al. 1991),

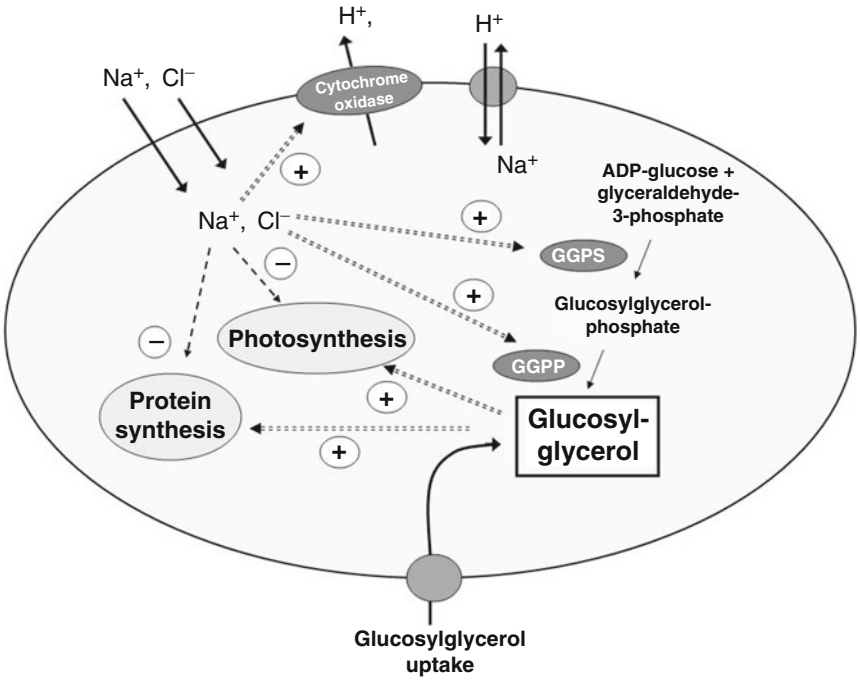

Fig. 15.3 Schematic drawing summarizing events which occur in cells of *Synechocystis* sp. PCC 6803 immediately after a hyperosmotic salt shock. *GGPS* glucosylglycerol-phosphate synthase, *GGPP* glucosylglycerol-phosphate phosphatase, +: ion-stimulated process, −: ion-inhibited process (Adapted from Hagemann et al. 1999)

different *Synechococcus* and *Synechocystis* isolates (Borowitzka et al. 1980; Richardson et al. 1983), *Spirulina platensis* (Warr et al. 1985), *Agmenellum quadruplicatum* (Tel-Or et al. 1986), and *Microcystis firma* (Erdmann et al. 1992). Large concentrations of glucosylglycerol were detected in natural communities of *M. chthonoplastes* in Lake Sivash, East Crimea (Zavarzin et al. 1993) and in a hypersaline microbial mat derived from Solar Lake, Sinai (Oren et al. 1994).

Intracellular concentrations of glucosylglycerol can be very high. In *Microcoleus* grown in 140 $g\,l^{-1}$ salt, glucosylglycerol accounted for 30% of the cell dry weight (Zavarzin et al. 1993). Upon increase of salinity by hyperosmotic shock, additional glucosylglycerol was produced. In the marine strain *Synechococcus* N100, CO_2 was the main carbon source for glucosylglycerol biosynthesis induced following an increase of medium NaCl concentration from 0.25 to 0.75 M. However, up to 10% may be derived from available intracellular organic carbon reserves. In the dark, no increase in glucosylglycerol was observed (Mackay and Norton 1987). Synthesis of glucosylglycerol was a slow process, and was only the final phase in a series of events which occur during salt upshock. Multiphasic osmotic adjustment in the euryhaline *Synechocystis* PCC 6714 upon a sudden salinity increase involves rapid entry of NaCl, followed by exchange of sodium by potassium ions. Many hours of adaptation are then required for the completion of glucosylglycerol accumulation to the proper new level in osmotic balance with the new higher salinity (Reed et al. 1985). Upon salt downshock, *Synechocystis* PCC 6714 released part of the excess glucosylglycerol to the medium (Fulda et al. 1990). Up to 90% of the low-molecular weight compounds were excreted after hypoosmotic shock. This hypoosmotically induced exudation was attributed to transient changes in nonspecific membrane permeability (Reed et al. 1986). However, in the marine *Agmenellum quadruplicatum* salt downshock induced increased carbohydrate turnover, and excess glucosylglycerol was probably metabolized to glycogen (Tel-Or et al. 1986).

Biosynthesis of glucosylglycerol was studied in depth in *Synechocystis* PCC 6803 (Hagemann 2010). This strain was isolated from a freshwater environment, but it grows well at salt concentrations two to three times as high as those of seawater (Richardson et al. 1983), and may thus be classified as a halotolerant euryhaline strain (Erdmann et al. 1992; Richardson et al. 1983). Intracellular glucosylglycerol concentrations were relatively low in cells grown in the presence of 0.65 and 1.03 M NaCl – 45 and 92 mM – respectively, and apparent intracellular sodium and potassium concentrations were high (Hagemann et al. 1994). Glucosylglycerol is synthesized from ADP-glucose and glycerol-3-phosphate with glucosylglycerol phosphate as an intermediate, in a two step reaction mediated by glucosylglycerol-phosphate synthase (gene *ggpS*) and glucosylglycerol-phosphate-phosphatase (gene *stpA*) (Hagemann et al. 1998) (Fig. 15.3). An assay for the glucosylglycerol phosphate phosphatase was developed (Schoor et al. 1996), and the *stpA* gene was characterized on the molecular level (Hagemann et al. 1997b). The enzyme system requires activation; in vivo by hypertonic salt concentrations (>100 mM) and in vitro by NaCl addition at the stage of enzyme extraction or assay. Salts such as KCl or $NaNO_3$ were less effective than NaCl as activators, and

organic osmolytes were inactive (Hagemann and Erdmann 1994). The activation of glucosylglycerol biosynthesis took also place in presence of chloramphenicol, and thus was not dependent on the synthesis of new proteins (Hagemann et al. 1990). The glucosylglycerol-phosphate synthase appears to be the main target for ion-mediated regulation of osmolyte synthesis (Schoor et al. 1999). Salt-stressed and salt-adapted cells show an about threefold increase in transcripts for *stpA* and *ggpS*, so regulation is both on the level of gene expression and on the level of the enzyme itself (Hagemann et al. 1999).

Mutants of *Synechocystis* PCC 6803 were isolated that are unable to grow at elevated salt concentrations. Three such mutants were obtained by random cartridge mutagenesis, and one of these was shown to be defective in glucosylglycerol synthesis (Hagemann and Zuther 1992). The mutant acquired salt resistance by taking up exogenous glucosylglycerol from the medium (Mikkat et al. 1996). This mutant, which tolerated up to 250 mM NaCl only, was found to be defective in the enzyme glucosylglycerol-phosphate phosphatase, and accumulated glucosylglycerol-phosphate intracellularly (Hagemann et al. 1997b). The latter compound was not effective as osmoprotectant, and may be toxic (Hagemann et al. 1996).

Synechocystis PCC 6803 has an active transport system for glucosylglycerol that permits uptake of glucosylglycerol from the medium. Uptake activity was enhanced in cells adapted to increasing concentrations of NaCl. Uptake was energy dependent, and was inhibited by uncouplers. The affinity of the uptake system was relatively low (K_m ~ 50 μM). Only sucrose and trehalose were found to compete for the transport system. The glucosylglycerol transport protein is encoded by the gene *ggtA*. Transcription of this gene is increased in cells adapted to high salt concentrations. When mutant strains impaired in this gene were grown in high salt media, significant amounts of glucosylglycerol were found in the medium, indicating that the transport system is mainly necessary for recovery of glucosylglycerol that had leaked through the cytoplasmic membrane (Hagemann et al. 1997a, 1998; Mikkat et al. 1996, 1997). Transport systems for the osmoprotective compounds trehalose and sucrose were also identified (Mikkat et al. 1997).

At least nine specific proteins were found at increased levels in *Synechococcus* PCC 6803 when grown at high salinities; the levels of a few other proteins were depressed (Hagemann et al. 1994). Upon salt shock the rate of protein synthesis dropped at first. Some proteins were then transiently synthesized during the first hours of the adaptation phase, while others remained to be synthesized at enhanced rates in salt-adapted cells (Hagemann et al. 1991). A number of salt-induced periplasmic proteins from *Synechocystis* sp. PCC 6803 were isolated and partially characterized (Fulda et al. 1999).

15.6.2.3 Glycine Betaine

Glycine betaine is characteristically found as an osmotic solute in the most salt tolerant of the cyanobacteria. Examples of strains that accumulate this compound include the *Aphanothece halophytica/Euhalothece* group (Reed et al. 1984a), *Spirulina subsalsa* from Bardawil Lagoon in Sinai, Egypt, an environment with fluctuating salinity (Gabbay-Azaria et al. 1988), *Dactylococcopsis salina* (Moore et al. 1987), and *Synechocystis* DUN52 (Reed et al. 1984a). In the last-named strain, isolated from calcareous stromatolites of intertidal flats in Kuwait, intracellular glycine betaine concentrations as high as 1.2, 2.4, and 3.0 M were measured in cells grown at 60, 100 and 200 $g\,l^{-1}$ salt, respectively (Mohammad et al. 1983). Glycine betaine was detected in massive amounts in the *Oscillatoria* mat covering the bottom of the hypersaline sulphur spring of Hamei Mazor (Sect. 15.5), using proton NMR spectroscopy (Oren et al. 1994). In most cases glycine betaine was found as the sole organic osmotic solute, but a *Gloeocapsa* strain had both glycine betaine and trehalose (Mackay et al. 1984). *Synechocystis* DUN52 contained minor amounts of glucosylglycerol in addition to glycine betaine (Mohammad et al. 1983).

Glycine betaine was very effective in protecting enzymatic activities against the inhibitory action of salt. Glycine betaine protected the structural integrity and activity of RUBISCO against the damaging influence of salts in *A. halophytica* (Incharoensakdi and Takabe 1988; Incharoensakdi et al. 1986). The glucose-6-phosphate dehydrogenase of *S. subsalsa* was markedly inhibited by salt (50% inhibition at 1.25 M NaCl), but in the presence of glycine betaine full activity was obtained at NaCl concentrations as high as 1.5 M (Gabbay-Azaria et al. 1988). In *Synechocystis* DUN52 KCl was stimulatory for glutamine synthetase at concentrations up to 1.3 M, but was inhibitory above 1.4 M. Glycine betaine did not inhibit up to 1.8–2 M, and the presence of 1 M betaine alleviated NaCl inhibition by 10–30% at NaCl concentrations between 0.8 and 2 M (Warr et al. 1984).

Upon a salt upshock from 0.5 to 1.5–2 M NaCl, plasmolysis occurred initially in *A. halophytica*, and electron micrographs showed disorganized thylakoid membranes. The rate of CO_2 fixation fell immediately, to return to normal levels within 1 day after synthesis of sufficient quantities of glycine betaine. The adaptation process was light-dependent, and no betaine accumulated in the dark (Ishitani et al. 1993).

In the past it was assumed that halophilic cyanobacteria synthesize glycine betaine from choline in a pathway resembling that found in higher plants (Hagemann et al. 1998). However it is now clear that glycine is the precursor in *A. halophytica* and in *Synechococcus* WH8102. In *A. halophytica*, two *N*-methyltransferases were characterized. One gene product catalyzed methylation of glycine and sarcosine with *S*-adenosylmethionine as the methyl donor; the second was responsible for the methylation of dimethylglycine to betaine.

The final product betaine did not cause feed-back inhibition even when present at the concentration of 2 M. Immunoblot analysis showed the accumulation of both enzymes to increase with increasing salinity (Waditee et al. 2003). *N*-methyltransferase genes involved in betaine synthesis were also characterised in the slightly halophilic *Synechococcus* WH8102, an organism that grows up to 2.8 $g\,l^{-1}$ salt. When cultures growing at 75% seawater salinity were supplemented with an additional 20 $g\,l^{-1}$ NaCl, the glycine betaine content increased from 7 to 62 mg g^{-1} dry weight. The genes coding for glycine sarcosine *N*-methyltransferase and sarcosine dimethylglycine *N*-methyltransferase were identified and overexpressed in *E. coli* (Lu et al. 2006).

Cyanobacteria that produce glycine betaine are also able to accumulate glycine betaine from the outside medium by means of an active transport system. Such transport activities were detected in *A. halophytica, Dactylococcopsis salina, Synechococcus* PCC 7418, and *Synechocystis* DUN 52. The transport serves as a scavenging system for exogenous glycine betaine, and use of glycine betaine present in the medium may be an effective strategy in environments of fluctuating salinity (Moore et al. 1987). Glycine betaine transport was not found in halophilic cyanobacteria that accumulate sucrose or glucosylglycerol. *A. halophytica* contains a betaine transporter active at alkaline pH and high salinity that is probably energized by Na^+-symport. Choline, sarcosine, and dimethylglycine did not compete for uptake of betaine (Laloknam et al. 2006). Salt stress also enhanced choline uptake in *A. halophytica*. The K_m of the inducible transport system was about 0.28 mM, and the V_{max} in salt-stressed cells (2 M NaCl) was twice that measured under unstressed conditions (0.5 M). The content of a periplasmic choline binding protein was higher in cells grown under salt stress. Choline stimulated growth under salt stress, but less so than glycine betaine (Incharoensakdi and Karnchanatat 2003). In *Synechocystis* DUN52, 1 h of incubation with 1 mM glycine betaine resulted in an intracellular concentration of 250 mM. The transport system had a K_m of 2 μM for glycine betaine, a V_{max} of 45 nmol min^{-1} mm^{-3} cell volume, and was optimally active at pH 8–8.5. Sodium chloride concentrations above 80 mM were required for stimulation of the transport activity. Severe hyperosmotic stress (1 M NaCl) reduced the rate of glycine betaine uptake but increased the internal betaine concentration (Moore et al. 1987).

Upon salt downshock excess glycine betaine may be excreted from the cells and released into the surrounding medium (Moore et al. 1987). The release may be due to transient permeability changes of the cell membrane, allowing leakage of low molecular weight metabolites (Reed et al. 1986). However, in *A. halophytica* a slow decrease (within 10 h) of intracellular glycine betaine concentrations was observed upon dilution of the medium from 1.5 to 0.5 M NaCl without any release to the outside medium (Ishitani et al. 1993). After downshock from 2 to 0.5 M NaCl, about half of intracellular glycine betaine was degraded after 36 h. Degradation was mediated by an inducible betaine-homocysteine methyltransferase. Some activity of this enzyme was also detected following carbon and nitrogen starvation (Incharoensakdi and Waditee 2000). The purified betaine-homocysteine methyltransferase was an octamer of 45 kDa subunits. The K_m for glycine betaine and homocysteine was 4.3 and 1.3 mM, respectively. Dimethylglycine and methionine are the products. The enzyme is inactivated by NaCl concentrations above 200 mM (Waditee and Incharoensakdi 2001).

15.6.2.4 Mycosporine-Like Amino Acids as Osmotic Solutes?

Large concentrations of mycosporine-like amino acids (MAAs) were found in a community of unicellular cyanobacteria that inhabit a gypsum crust developing on the bottom of a hypersaline saltern pond in Eilat, Israel (Oren et al. 1995; Sect. 15.3.4). Two MAAs were detected, one with an absorption maximum at 332 nm and one at 365 nm. The first was identified as mycosporine-2-glycine (Kedar et al. 2002), and the structure of the second, a novel compound ("euhalothece-362"), was elucidated as well (Volkmann et al. 2006). Intracellular MAA concentrations in the cyanobacterial community were estimated to be at least 98 mM, and this already high value is probably an underestimate. With an average molecular weight of around 300, MAAs should thus contribute at least 3% of the cell wet weight. While MAAs were shown to act as sunscreen compounds, protecting the cells against solar UV radiation (Garcia-Pichel and Castenholz 1993; Chap. 19), when occurring in such high concentrations they may also have an osmotic function and help the cells cope with the high salt concentrations of their environment. This agrees with the concept that MAAs may serve a number of additional functions in addition to UV screening (Oren and Gunde-Cimerman 2007). When material from the upper layer of the Eilat gypsum crust was subjected to slow dilution with distilled water, MAAs rapidly appeared in the outer medium and the extent of loss of intracellular MAAs was approximately proportional to the extent of the dilution stress applied (Oren 1997). The mechanism of release may again be related to transient permeability changes of the cell membrane, as suggested for glycine betaine release (Reed et al. 1986).

15.7 Biotechnological Applications of Halophilic Cyanobacteria

A number of interesting applications have been suggested for halophilic cyanobacteria, their products and their genes. *Aphanothece* releases massive amounts of slime (De Philippis et al. 1993; Jones and Yopp 1979; Li et al. 2001; Sudo et al. 1995;

Sect. 15.4.1). This polysaccharide slime, with xanthan-like properties, may have promising industrial uses (De Philippis et al. 1998; Morris et al. 2001). Therefore attempts have been made to optimize polysaccharide production using different cultivation systems, including cells immobilized on light-diffusing optical fibers. In such a system up to 116 mg polysaccharide was obtained mg^{-1} dry cell weight day^{-1} (Matsunaga et al. 1996). The finding that oral administration of *A. halophytica* exopolysaccharide in mice significantly inhibits influenza virus (H1N1)-induced pneumonia by modulating the host immune system also opens up interesting possibilities for applications (Zheng et al. 2006).

Several genes of *A. halophytica* have aroused interest in biotechnological applications. Its three types of restriction endonucleases (Whitehead and Brown 1982, 1985) may find applications in molecular biology research. Even more exciting is the prospect of expressing glycine betaine formation in higher plants to increase their salt tolerance. Expression of *Aphanothece* 3-phosphoglycerate dehydrogenase, which catalyzes the first step of the phosphorylated pathway of serine biosynthesis, in *E. coli* caused led to increased levels of betaine, as well as of glycine and serine. When the betaine biosynthesis pathway via glycine methylation was introduced into *Arabidopsis* plants in which, betaine levels were increased and stress tolerance improved (Waditee et al. 2007).

Finally, the DnaK chaperone of *Aphanothece*, when expressed in different plants, greatly increased their tolerance to different adverse conditions. Temperature tolerance of tobacco during germination and early growth was increased (Ono et al. 2001; Sugino et al. 1999), quantum yield of photosynthesis in young seedlings was increased, salt tolerance was improved (Sugino et al. 1999), and a higher seed yield was obtained (Uchida et al. 2008). Transgenic rice plants expressing the protein had an enhanced level of Calvin cycle enzymes, showed faster growth and higher seed yield, and an enhanced tolerance for high temperature and salt stress (Uchida et al. 2008). When expressed in poplar plants, faster growth was obtained under high light intensity, and the transgenic plants achieved larger size and higher cellulose content than control plants. They also recovered more rapidly from stress by high salinity, drought and low temperature (Takabe et al. 2008).

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References

Abed RMM, Garcia-Pichel F, Hernández-Mariné M (2002) Polyphasic characterization of benthic, moderately halophilic, moderately thermophilic cyanobacteria with very thin trichomes and the proposal of *Halomicronema excentricum* gen. nov., sp. nov. Arch Microbiol 177:361–370

Abed RMM, Polerecky L, Al Najjar M, de Beer D (2006) Effect of temperature on photosynthesis, oxygen consumption and sulfide production in an extremely hypersaline cyanobacterial mat. Aquat Microb Ecol 44:21–30

Abed RMM, Kohls K, Schoon R, Scherf A-K, Schacht M, Palinska KA, Al-Hassani H, Hamza RJ, Golubic S (2008) Lipid biomarkers, pigments and cyanobacterial diversity of microbial mats across intertidal flats of the arid coast of the Arabian Gulf (Abu Dhabi, UAE). FEMS Microbiol Ecol 65:449–462

Ali GH (1999) Phytoplankton activities in hypersaline, anoxic conditions. I. Dynamics of phytoplankton succession in the Solar Lake (Taba, Egypt). Water Sci Technol 40(7):117–125

Anderson GC (1958) Some limnological features of a shallow saline meromictic lake. Limnol Oceanogr 3:259–270

Arieli B, Binder B, Shahak Y, Padan E (1989) Sulfide induction of synthesis of a periplasmic protein in the cyanobacterium *Oscillatoria limnetica*. J Bacteriol 171:699–702

Arieli B, Padan E, Shahak Y (1991) Sulfide-induced sulfide-quinone reductase activity in thylakoids of *Oscillatoria limnetica*. J Biol Chem 266:104–111

Arieli B, Shahak Y, Taglicht D, Hauska G, Padan E (1994) Purification and characterization of sulfide-quinone reductase, a novel enzyme driving anoxygenic photosynthesis in *Oscillatoria limnetica*. J Biol Chem 269:5705–5711

Asami S, Takabe T, Akazawa T, Codd GA (1983) Ribulose 1,5-bisphosphate carboxylase from the halophilic cyanobacterium *Aphanothece halophytica*. Arch Biochem Biophys 225:713–721

Batterton JC, Van Baalen C (1971) Growth responses of blue-green algae to sodium chloride concentration. Arch Mikrobiol 76:151–165

Bauld J (1981) Occurrence of benthic microbial mats in saline lakes. Hydrobiologia 81:87–111

Bebout BM, Carpenter SP, Des Marais DJ, Discipulo M, Embaye T, Garcia-Pichel F, Hoehler TM, Hogan M, Jahnke LL, Keller RM, Miller SR, Prufert-Bebout LE, Raleigh C, Rothrock M, Turk K (2002) Long-term manipulations of intact microbial mat communities in a greenhouse collaboratory: simulating Earth's present and past field environments. Astrobiology 2:383–402

Berland B, Le Campion T, Campos Baeta Neves MH (1989) Interaction de la salinité et de la température sur la morphologie, la croissance et la composition cellulaire d'une Cyanobactérie halotolérante (*Aphanothece* sp.). Bot Mar 32:317–329

Blumwald E, Tel-Or E (1982) Osmoregulation and cell composition in salt-adaptation of *Nostoc muscorum*. Arch Microbiol 132: 168–172

Blumwald E, Mehlhorn RJ, Packer L (1983) Studies of osmoregulation in salt adaptation of cyanobacteria with ESR spin-probe techniques. Proc Natl Acad Sci USA 80:2599–2602

Borowitzka LJ (1981) The microflora. Adaptations to life in extremely saline lakes. Hydrobiologia 81:33–46

Borowitzka LJ, Demmerle S, Mackay MA, Norton RS (1980) Carbon-13 nuclear magnetic resonance study of osmoregulation in a blue-green alga. Science 210:650–651

Braithwaite CJR, Whitton BA (1987) Gypsum and halite associated with the cyanobacterium *Entophysalis*. Geomicrobiol J 5:43–55

Brock TD (1976) Halophilic blue-green algae. Arch Microbiol 107:109–111

Bronstein M, Schutz M, Hauska G, Padan E, Shashak Y (2000) Cyanobacterial sulfide-quinone reductase: cloning and heterologous expression. J Bacteriol 182:3336–3344

Burke CM (1995) Benthic microbial production of oxygen supersaturates the bottom water of a stratified hypersaline lake. Microb Ecol 29:163–171

Camacho A, de Wit R (2003) Effect of nitrogen and phosphorus additions on a benthic microbial mat from a hypersaline lake. Aquat Microb Ecol 32:261–273

Campbell SE, Golubic S (1985) Benthic cyanophytes (cyanobacteria) of Solar Lake (Sinai). Arch Hydrobiol 38/39(Suppl 71):311–329

Canfield DE, Des Marais DJ (1993) Biogeochemical cycles of carbon, sulfur, and free oxygen in microbial mat. Geochim Cosmochim Acta 57:3971–3984

Canfield DE, Sørensen KB, Oren A (2004) Biogeochemistry of a gypsum-encrusted microbial ecosystem. Geobiology 2:133–150

Casillas-Martinez L, Gonzalez ML, Fuentes-Figueroa Z, Castro CM, Nieves-Mendez D, Hernandez C, Ramirez W, Systsma RE, Perez-Jimenez J, Visscher PT (2005) Community structure, geochemical characteristics and mineralogy of a hypersaline microbial mat, Cabo Rojo, PR. Geomicrobiol J 22:269–281

Caumette P, Matheron R, Raymond N, Relexans J-C (1994) Microbial mats in the hypersaline ponds of Mediterranean salterns (Salins-de-Giraud, France). FEMS Microbiol Ecol 13:273–286

Cohen Y (1984a) The Solar Lake cyanobacterial mats: strategies of photosynthetic life under sulfide. In: Cohen Y, Castenholz RW, Halvorson HO (eds) Microbial mats: stromatolites. Alan R Liss, New York, pp 133–148, 498 pp

Cohen Y (1984b) Oxygenic photosynthesis, anoxygenic photosynthesis, and sulfate reduction in cyanobacterial mats. In: Klug MJ, Reddy CA (eds) Current perspectives in microbial ecology. American Society for Microbiology, Washington, DC, pp 435–441, 494 pp

Cohen Y, Krumbein WE, Shilo M (1975a) The Solar Lake: limnology and microbiology of a hypersaline, monomictic heliothermal heated sea-marginal pond (Gulf of Aqaba, Sinaï). Rapp Comm Int Mer Médit 23:105–107

Cohen Y, Padan E, Shilo M (1975b) Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. J Bacteriol 123:855–861

Cohen Y, Jørgensen BB, Padan E, Shilo M (1975c) Sulphide-dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. Nature 257:489–492

Cohen Y, Krumbein WE, Goldberg M, Shilo M (1977a) Solar Lake (Sinai). 1. Physical and chemical limnology. Limnol Oceanogr 22:597–608

Cohen Y, Krumbein WE, Shilo M (1977b) Solar Lake (Sinai). 2. Distribution of photosynthetic microorganisms and primary production. Limnol Oceanogr 22:609–620

Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R (1986) Adaptation to hydrogen sulfide of oxygenic and anoxygenic photosynthesis among cyanobacteria. Appl Environ Microbiol 51:398–407

Coleman MU, White MA (1993) The role of biological disturbances in the production of solar salt. In: Seventh symposium on salt, vol 1. Elsevier Science Publishers, Amsterdam, pp 623–631

Cornée A, Dickman M, Busson G (1992) Laminated cyanobacterial mats in sediments of solar salt works: some sedimentological implications. Sedimentology 39:599–612

D'Antoni D'Amelio E, Cohen Y, Des Marais DJ (1987) Association of a new type of gliding, filamentous, purple phototrophic bacterium inside bundles of *Microcoleus chthonoplastes* in hypersaline cyanobacterial mats. Arch Microbiol 147:213–220

D'Antoni D'Amelio E, Cohen Y, Des Marais DJ (1989) Comparative functional ultrastructure of two hypersaline submerged cyanobacterial mats: Guerrero Negro, Baja California Sur, Mexico, and Solar Lake, Sinai, Egypt. In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 97–113, 494 pp

Davis JS (1974) Importance of micro-organisms in solar salt production. In: Fourth symposium on salt, vol 1. Northern Ohio Geological Society, Cleveland, pp 369–372

Davis JS (1978) Biological communities in a nutrient enriched salina. Aquat Bot 4:23–42

Davis JS (1993) Biological management for problem solving and biological concepts for a new generation of solar saltworks. In: Kakihana H, Hardy HR Jr, Hoshi T, Toyokura K (eds) Proceedings of the seventh symposium on salt, vol 1. Elsevier Science Publishers, Amsterdam, pp 611–616

Davis JS, Giordano M (1996) Biological and physical events involved in the origin, effects, and control of organic matter in solar saltworks. Int J Salt Lake Res 4:335–347

De Medeiros Rocha R, Camara MR (1993) Prediction, monitoring and management of detrimental algal blooms on solar salt production in north-east Brazil. In: Seventh symposium on salt, vol 1. Elsevier Science Publishers, Amsterdam, pp 657–660

De Philippis R, Margheri MC, Pelosi E, Ventura S (1993) Exopolysaccharide production by a unicellular cyanobacterium isolated from a hypersaline habitat. J Appl Phycol 5:387–394

De Philippis R, Margheri MC, Materassi R, Vincenzini M (1998) Potential of unicellular cyanobacteria from saline environments as exopolysaccharide producers. Appl Environ Microbiol 64:1130–1132

de Wit R, van Boekel WHM, van Gemerden H (1988) Growth of the cyanobacterium *Microcoleus chthonoplastes* on sulfide. FEMS Microbiol Ecol 53:203–209

de Wit R, Falcón LI, Carpy-Roubaud C (2005) Heterotrophic dinitrogen fixation (acetylene reduction) in phosphate-fertilised *Microcoleus chthonoplastes* microbial mat from the hypersaline inland lake 'la Salada de Chiprana' (NE Spain). Hydrobiologia 534:245–253

Des Marais DJ (1995) The biogeochemistry of hypersaline microbial mats. In: Jones JG (ed) Advances in microbial ecology, vol 14. Plenum Press, New York, pp 251–274, 416 pp

Dor I, Hornoff M (1985) Studies on *Aphanothece halophytica* Frémy from a solar pond: comparison of two isolates on the basis of cell polymorphism and growth response to salinity, temperature and light conditions. Bot Mar 28:389–398

Dubinin AV, Gerasimenko LM (1993) Dark anaerobic metabolism of halophilic cyanobacterium *Microcoleus chthonoplastes*. Mikrobiologiya (English translation) 62:391–394

Dubinin AV, Gerasimenko LM, Gusev MV (1992a) Physiological features of a strain of *Microcoleus chthonoplastes* from a hypersaline reservoir. Mikrobiologiya (English translation) 61:47–51

Dubinin AV, Zastrizhnaya OM, Gusev MV (1992b) Hydrogen peroxide production by the halophilic cyanobacterium *Microcoleus chthonoplastes*. Mikrobiologiya (English translation) 61:261–266

Ehrlich A, Dor I (1985) Photosynthetic microorganisms of the Gavish sabkha. In: Friedman GM, Krumbein WE (eds) Hypersaline ecosystems. The Gavish Sabkha. Springer, New York, pp 296–321, 484 pp

Erdmann N, Fulda S, Hagemann M (1992) Glucosylglycerol accumulation during salt acclimation of two unicellular cyanobacteria. J Gen Microbiol 138:363–368

Estrada M, Henriksen P, Gasol JM, Casamayor EO, Pedrós-Alió C (2004) Diversity of planktonic photoautotrophic microorganisms along a salinity gradient as depicted by microscopy, flow cytometry, pigment analysis and DNA-based methods. FEMS Microbiol Ecol 49:281–293

Felix EA, Rushforth SR (1979) The algal flora of the Great Salt Lake, Utah, U.S.A. Nova Hedwig 31:163–195

Fourçans A, Solé A, Dienstra E, Ranchou-Peyruse A, Esteve I, Caumette P, Duran R (2006) Vertical migration of phototrophic bacterial populations in a hypersaline microbial mat from Salins-de-Giraud (Camargue, France). FEMS Microbiol Ecol 57:367–377

Fulda S, Hagemann M, Libbert E (1990) Release of glucosylglycerol from the cyanobacterium *Synechocystis* spec. SAG 92.79 by hypoosmotic shock. Arch Microbiol 153:405–408

Fulda S, Mikkat S, Schröder W, Hagemann M (1999) Isolation of salt-induced periplasmic proteins from *Synechocystis* sp. strain PCC 6803. Arch Microbiol 171:214–217

Gabbay-Azaria R, Tel-Or E (1991) Regulation of intracellular Na^+ content during NaCl upshock in the marine cyanobacterium *Spirulina subsalsa* cells. Biores Technol 38:215–220

Gabbay-Azaria R, Tel-Or E (1993) Mechanisms of salt tolerance in cyanobacteria. In: Gresshoff PM (ed) Plant responses to the environment. CRC Press, Boca Raton, pp 123–132, 208 pp

Gabbay-Azaria R, Tel-Or E, Schonfeld M (1988) Glycinebetaine as an osmoregulant and compatible solute in the marine cyanobacterium *Spirulina subsalsa*. Arch Biochem Biophys 264:333–339

Gabbay-Azaria R, Schonfeld M, Tel-Or S, Messinger R, Tel-Or E (1992) Respiratory activity in the marine cyanobacterium *Spirulina subsalsa* and its role in salt tolerance. Arch Microbiol 157:183–190

Garcia-Pichel F, Castenholz RW (1993) Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl Environ Microbiol 59:163–169

Garcia-Pichel F, Prufert-Bebout L, Muyzer G (1996) Phenotypic and phylogenetic analysis show *Microcoleus chthonoplastes* to be a cosmopolitic cyanobacterium. Appl Environ Microbiol 62:3284–3291

Garcia-Pichel F, Nübel U, Muyzer G (1998) The phylogeny of unicellular, extremely halotolerant cyanobacteria. Arch Microbiol 169:469–482

Garlick S, Oren A, Padan E (1977) Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. J Bacteriol 129:623–629

Gerasimenko LM, Mityushina LL, Namsaraev BB (2003) *Microcoleus* mats from alkaliphilic and halophilic communities. Mikrobiologiya (English translation) 72:71–79

Gerdes G, Krumbein WE, Holtkamp S (1985) Salinity and water activity related zonation of microbial communities and potential stromatolites of the Gavish sabkha. In: Friedman GM, Krumbein WE (eds) Hypersaline ecosystems. The Gavish Sabkha. Springer, New York, pp 169–183, 484 pp

Giani D, Seeler J, Giani L, Krumbein WE (1989) Microbial mats and physicochemistry in a saltern in the Bretagne (France) and in a laboratory scale saltern model. FEMS Microbiol Ecol 62:151–162

Giri BJ, Bano N, Hollibaugh JT (2004) Distribution of RuBisCO genotypes along a redox gradient in Mono Lake, California. Appl Environ Microbiol 70:3443–3448

Goldman CR, Mason DT, Hobbie JE Jr (1967) Two Antarctic desert lakes. Limnol Oceanogr 12:295–310

Golubic S (1980) Halophily and halotolerance in cyanophytes. Orig Life 10:169–183

Green SJ, Blackford C, Bucki P, Jahnke LL (2008) A salinity and sulfate manipulation of hypersaline microbial mats reveals stasis in the cyanobacterial community structure. ISME J 2:457–470

Grimalt JO, de Wit R, Teixidor P, Albaiges J (1992) Lipid biogeochemistry of *Phormidium* and *Microcoleus* mats. Org Geochem 19:509–530

Hagemann M (2010) Molecular biology of cyanobacterial salt acclimation. FEMS Microbiol Rev 35(1):87–123. doi:10.1111/j.1574-6976.2010.000234.x

Hagemann M, Erdmann N (1994) Activation and pathway of glucosylglycerol synthesis in the cyanobacterium *Synechocystis* sp. PCC 6803. Microbiology 140:1427–1431

Hagemann M, Zuther E (1992) Selection and characterization of mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 unable to tolerate high salt concentrations. Arch Microbiol 158:429–434

Hagemann M, Wölfel L, Krüger B (1990) Alterations of protein synthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 after a salt shock. J Gen Microbiol 136:1393–1399

Hagemann M, Techel D, Rensing L (1991) Comparison of salt- and heat-induced alterations of protein synthesis in the cyanobacterium *Synechocystis* sp. PCC 6803. Arch Microbiol 155:587–592

Hagemann M, Fulda S, Schubert H (1994) DNA, RNA, and protein synthesis in the cyanobacterium *Synechocystis* sp. PCC 6803 adapted to different salt concentrations. Curr Microbiol 28:201–207

Hagemann M, Richter S, Zuther E, Schoor A (1996) Characterization of a glucosylglycerol-phosphate-accumulating, salt sensitive mutant of the cyanobacterium *Synechocystis* sp. strain PCC 6803. Arch Microbiol 166:83–91

Hagemann M, Richter S, Mikkat (1997a) The *ggtA* gene encodes a subunit of the transport system for the osmoprotective compound glucosylglycerol in *Synechocystis* sp. strain PCC 6803. Arch Microbiol 166:83–91

Hagemann M, Schoor A, Jeanjean R, Zuther E, Joset F (1997b) The *stpA* gene from *Synechocystis* sp. strain PCC 6803 encodes the glucosylglycerol-phosphate phosphatase involved in cyanobacterial osmotic response to salt shock. J Bacteriol 179:1727–1733

Hagemann M, Schoor A, Mikkat S, Effmert U, Zuther E, Marin K, Fulda S, Vinnemeyer J, Kunert A, Milkowski C, Probst C, Erdmann N (1998) The biochemistry and genetics of the synthesis of osmoprotective compounds in cyanobacteria. In: Oren A (ed) Microbiology and biogeochemistry of hypersaline environments. CRC Press, Boca Raton, pp 177–186, 359 pp

Hagemann M, Schoor A, Effmert U, Zuther E, Marin K, Erdmann N (1999) The biochemistry and genetics of the glucosylglycerol synthesis in *Synechocystis* sp. PCC 6803. In: Peschek GA, Löffelhardt W, Schmetterer G (eds) The phototrophic prokaryotes. Kluwer Academic/Plenum Publishers, New York, pp 583–587, 836 pp

Hof T, Frémy P (1933) On Myxophyceae living in strong brines. Rec Trav Bot Néerl 30:140–162

Imhoff JF, Sahl HG, Soliman GSH, Trüper HG (1979) The Wadi Natrun: chemical composition and microbial mass developments in alkaline brines of eutrophic desert lakes. Geomicrobiol J 1:219–234

Incharoensakdi A, Karnchanatat A (2003) Salt stress enhances choline uptake in the halotolerant cyanobacterium *Aphanothece halophytica*. Biochim Biophys Acta 1621:102–109

Incharoensakdi A, Kitjaharn P (2002) Zinc biosorption from aqueous solution by a halotolerant cyanobacterium *Aphanothece halophytica*. Curr Microbiol 45:261–264

Incharoensakdi A, Laloknam S (2005) Nitrate uptake in the halotolerant cyanobacterium *Aphanothece halophytica* is energy-dependent driven by ΔpH. J Biochem Mol Biol 38:468–473

Incharoensakdi A, Takabe T (1988) Determination of intracellular chloride ion concentration in a halotolerant cyanobacterium *Aphanothece halophytica*. Plant Cell Physiol 29:1073–1075

Incharoensakdi A, Waditee R (2000) Degradation of glycinebetaine by betaine-homocysteine methyltransferase in *Aphanothece halophytica*: effect of salt downshock and starvation. Curr Microbiol 41:227–231

Incharoensakdi A, Takabe T, Akazawa T (1986) Effect of betaine on enzyme activity and subunit interaction of ribulose-1,5-bisphosphate carboxylase/oxygenase from *Aphanothece halophytica*. Plant Physiol 81:1044–1049

Ionescu D, Lipski A, Altendorf K, Oren A (2007) Characterization of the endoevaporitic microbial communities in a hypersaline gypsum crust by fatty acid analysis. Hydrobiologia 576:15–26

Ishitani M, Takabe T, Kojima K, Takabe T (1993) Regulation of glycinebetaine accumulation in the halotolerant cyanobacterium *Aphanothece halophytica*. Aust J Plant Physiol 20:693–703

Jahnke LL, Lee B, Sweeney MJ, Klein HP (1989) Anaerobic biosynthesis of unsaturated fatty acids in the cyanobacterium, *Oscillatoria limnetica*. Arch Microbiol 152:215–217

Javor B (1989) Hypersaline environments. Microbiology and biogeochemistry. Springer, Berlin, p 328

Javor BJ (2002) Industrial microbiology of solar salt production. J Ind Microbiol Biotechnol 28:42–47

Jones JH, Yopp JH (1979) Cell wall constituents of *Aphanothece halophytica* (Cyanophyta). J Phycol 15:62–66

Jones AG, Ewing CM, Melvin MV (1981) Biotechnology of solar saltfields. Hydrobiologia 82:391–406

Jonkers HM, Ludwig R, de Wit R, Pringault O, Muyzer G, Niemann H, Finke N, de Beer D (2003) Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: 'La Salada de Chiprana' (NE Spain). FEMS Microbiol Ecol 44:175–189

Jørgensen BB, Kuenen JG, Cohen Y (1979) Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). Limnol Oceanogr 24:799–822

Jørgensen BB, Revsbech NP, Cohen Y (1983) Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. Limnol Oceanogr 28:1075–1093

Jørgensen BB, Cohen Y, Revsbech NP (1986) Transition from anoxygenic to oxygenic photosynthesis in a *Microcoleus chthonoplastes* cyanobacterial mat. Appl Environ Microbiol 51:408–417

Joset P, Jeanjean R, Hagemann M (1996) Dynamics of the response of cyanobacteria to salt stress: deciphering of molecular events. Physiol Plant 96:738–744

Kao OHW, Berns DS, Town WR (1973) The characterization of C-phycocyanin from an extremely halo-tolerant blue-green alga, *Coccochloris elabens*. Biochem J 131:39–50

Karsten U (1996) Growth and organic osmolytes of geographically different isolates of *Microcoleus chthonoplastes* (*Cyanobacteria*) from benthic microbial mats: response to salinity change. Phycology 32:501–506

Karsten U, Garcia-Pichel F (1996) Carotenoids and mycosporine-like amino acid compounds in members of the genus *Microcoleus* (Cyanobacteria): a chemosystematic study. Syst Appl Microbiol 19:285–294

Kedar L, Kashman Y, Oren A (2002) Mycosporine-2-glycine is the major mycosporine-like amino acid in a unicellular cyanobacterium (*Euhalothece* sp.) isolated from a gypsum crust in a hypersaline saltern pond. FEMS Microbiol Lett 208:233–237

Kevbrin VV, Dubinin AV, Osipov GA (1991) Osmoregulation in the marine cyanobacterium *Microcoleus chthonoplastes*. Mikrobiologiya (English translation) 60:407–410

Kirkwood AE, Buchheim JA, Buchheim MA, Henley WJ (2008) Cyanobacterial diversity and halotolerance in a variable hypersaline environment. Microb Ecol 55:453–465

Kohls K, Abed RMM, Polerecky L, Weber M, de Beer D (2010) Halotaxis of cyanobacteria in an intertidal hypersaline microbial mat. Environ Microbiol 12:567–575

Komárek J (1976) The taxonomic review of the genera *Synechocystis* Sauv. 1892, *Synechococcus* Näg. 1849, and *Cyanothece* gen. nov. (*Cyanophyceae*). Arch Protistenkd 118:119–179

Komárek J, Anagnostidis K (1995) Nomenclatural novelties in chroococcalean cyanoprokaryotes. Preslia 67:15–23

Komárek J, Cepák V (1998) Cytomorphological characters supporting the taxonomic validity of *Cyanothece* (*Cyanoprokaryota*). Plant Syst Evol 210:25–39

Komárek J, Cepák V, Kaštovský J, Sulek J (2004) What are the cyanobacterial genera *Cyanothece* and *Cyanobacterium*? Contribution to the combined molecular and phenotype taxonomic evaluation of cyanobacterial diversity. Arch Hydrobiol Suppl 153:1–36

Krumbein WE, Cohen Y, Shilo M (1977) Solar Lake (Sinai). 4. Stromatolitic cyanobacterial mats. Limnol Oceanogr 22:635–656

Laloknam S, Tanaka K, Buaboocha T, Waditee R, Incharoensakdi A, Hibino T, Tanaka Y, Takabe T (2006) Halotolerant cyanobacterium *Aphanothece halophytica* contains a betaine transporter active at alkaline pH and high salinity. Appl Environ Microbiol 72:6018–6026

Lee BH, Hibino T, Jo J, Viale AM, Takabe T (1997) Isolation and characterization of a *dnaK* genomic locus in a halotolerant cyanobacterium *Aphanothece halophytica*. Plant Mol Biol 35:763–775

Li P, Liu Z, Xu R (2001) Chemical characterization of the released polysaccharide from the cyanobacterium *Aphanothece halophytica*. J Appl Phycol 13:71–77

Lu W-D, Chi Z-M, Su C-D (2006) Identification of glycine betaine as compatible solute in *Synechococcus* sp. WH8102 and characterization of its *N*-methyltransferase genes involved in betaine synthesis. Arch Microbiol 186:495–506

Mackay MA, Norton RS (1987) ^{13}C nuclear magnetic resonance study of biosynthesis of glucosylglycerol by a cyanobacterium under osmotic stress. J Gen Microbiol 133:1535–1542

Mackay MA, Norton RS, Borowitzka LJ (1984) Organic osmoregulatory solutes in cyanobacteria. J Gen Microbiol 130:2177–2191

Mancinelli RL, White MR, Rothschild LJ (1998) Biopan-survival I: exposure of the osmophiles *Synechococcus* sp. (Nägeli) and *Haloarcula* sp. to the space environment. Adv Space Res 22:327–334

Marcarelli AM, Wurtsbaugh WA, Griset O (2006) Salinity controls phytoplankton response to nutrient enrichment in the Great Salt Lake, Utah, USA. Can J Fish Aquat Sci 63:2236–2248

Margheri MC, Tredici MR, Barsanti L, Balloni W (1987) The photosynthetic community of the Trapani saline lagoons: an alternative option for the exploitation of an extreme environment. Ann Microbiol 37:203–215

Margheri MC, Bosco M, Giovannetti L, Ventura S (1999) Assessment of the genetic diversity of halotolerant coccoid cyanobacteria using amplified 16S rDNA restriction analysis. FEMS Microbiol Lett 173:9–16

Margheri MC, Ventura S, Kaštovský J, Komárek J (2008) The taxonomic validation of the cyanobacterial genus *Halothece*. Phycologia 47:477–486

Matsunaga T, Sudo H, Takemasa H, Wachi Y, Nakamura N (1996) Sulfated extracellular polysaccharide production by the halophilic cyanobacterium *Aphanocapsa halophytica* immobilized on light-diffusing optical fibers. Appl Microbiol Biotechnol 45:24–27

Mikhoudyuk OS, Zavarzin GA, Ivanovsky RN (2008) Transport systems for carbonate in the extremely natronophilic cyanobacterium *Euhalothece* sp. Mikrobiologiya (English translation) 77:412–418

Mikkat S, Hagemann M, Schoor A (1996) Active transport of glucosylglycerol is involved in salt adaptation of the cyanobacterium *Synechocystis* sp. strain PCC 6803. Microbiology 142:1725–1732

Mikkat S, Effmert U, Hagemann M (1997) Uptake and use of the osmoprotective compounds trehalose, glucosylglycerol, and sucrose by the cyanobacterium *Synechococcus* sp. PCC 6803. Arch Microbiol 167:112–118

Miller DM, Jones JH, Yopp JH, Tindall RD, Schmid WE (1976) Ion metabolism in a halophilic blue-green alga, *Aphanothece halophytica*. Arch Microbiol 111:145–149

Moezelaar R, Bijvank SM, Stal LJ (1996) Fermentation and sulfur reduction in the mat building cyanobacterium *Microcoleus chthonoplastes*. Appl Environ Microbiol 62:1752–1758

Mohammad FAA, Reed RH, Stewart WDP (1983) The halophilic cyanobacterium *Synechocystis* DUN52 and its osmotic responses. FEMS Microbiol Lett 16:287–290

Moore DJ, Reed RH, Stewart WDP (1987) A glycine betaine transport system in *Aphanothece halophytica* and other glycine betaine-synthesising cyanobacteria. Arch Microbiol 147:399–405

Morris GA, Li P, Puaud M, Liu Z, Mitchell JR, Harding SE (2001) Hydrodynamic characterization of the exopolysaccharide from the halophilic cyanobacterium *Aphanothece halophytica* GR02: a comparison with xanthan. Carbohydr Res 44:261–268

Nagasathya A, Thajuddin N (2008) Cyanobacterial diversity in the hypersaline environment of the saltpans of southeastern coast of India. Asian J Plant Sci 7:473–478

Nübel U, Garcia-Pichel F, Kühl M, Muyzer G (1999) Spatial scale and the diversity of benthic cyanobacteria and diatoms in a salina. Hydrobiologia 401:199–206

Nübel U, Garcia-Pichel F, Clavero E, Muyzer G (2000a) Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. Environ Microbiol 2:217–226

Nübel U, Garcia-Pichel F, Muyzer G (2000b) The halotolerance and phylogeny of cyanobacteria with tightly coiled trichomes (*Spirulina* Turpin) and the description of *Halospirulina tapeticola* gen. nov., sp. nov. Int J Syst Evol Microbiol 50:1265–1277

Ono K, Hibino T, Kohinata T, Suzuki S, Tanaka Y, Nakamura T, Takabe T, Takabe T (2001) Overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytica* enhances the high-temperature tolerance of tobacco during germination and early growth. Plant Sci 160:455–461

Oren A (1988) The microbial ecology of the Dead Sea. In: Marshall KC (ed) Advances in microbial ecology, vol 10. Plenum Press, New York, pp 193–229, 447 pp

Oren A (1989) Photosynthetic and heterotrophic benthic bacterial communities of a hypersaline sulfur spring on the shore of the Dead Sea (Hamei Mazor). In: Cohen Y, Rosenberg E (eds) Microbial mats. Physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 64–76, 499 pp

Oren A (1997) Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. Geomicrobiol J 14:233–242

Oren A (2002) Halophilic microorganisms and their environments. Springer, Dordrecht, p 575

Oren A (2005) Microscopic examination of microbial communities along a salinity gradient in saltern evaporation ponds: a 'halophilic safari'. In: Gunde-Cimerman N, Oren A, Plemenitaš A (eds) Adaptation to life at high salt concentrations in archaea, bacteria, and eukarya. Springer, Dordrecht, pp 41–57, 573 pp

Oren A (2006) Life at high salt concentrations. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology and biochemistry, vol 2. Springer, New York, pp 263–282, 1107 pp

Oren A (2007) Diversity of organic osmotic compounds and osmotic adaptation in cyanobacteria and algae. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 641–655, 811 pp

Oren A (2009a) Problems associated with the taxonomic validation of the cyanobacterial genus *Halothece* (Margheri et al 2008, Phycologia 47:477–486). Phycologia 48:313–314

Oren A (2009b) Saltern evaporation ponds as model systems for the study of primary production processes under hypersaline conditions. Aquat Microb Ecol 56:193–204

Oren A, Gunde-Cimerman N (2007) Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? FEMS Microbiol Lett 269:1–10

Oren A, Padan E (1978) Induction of anaerobic, photoautotrophic growth in the cyanobacterium *Oscillatoria limnetica*. J Bacteriol 133:558–563

Oren A, Seckbach J (2001) Oxygenic photosynthetic microorganisms in extreme environments. In: Elster J, Seckbach J, Vincent W, Lhotsky O (eds) Algae and extreme environments – ecology and physiology. Nova Hedwigia Beih 123:13–31

Oren A, Shilo M (1979) Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica*: sulfur respiration and lactate fermentation. Arch Microbiol 122:77–84

Oren A, Tindall BJ (2005) Nomenclature of the cyanophyta/cyanobacteria/cyanoprokaryotes under the International Code of Nomenclature of Prokaryotes. Algol Stud 117; Arch Hydrobiol Suppl 159:39–52

Oren A, Padan E, Avron M (1977) Quantum yields for oxygenic and anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. Proc Natl Acad Sci USA 74:2152–2156

Oren A, Padan E, Malkin S (1979) Sulfide inhibition of photosystem I in cyanobacteria (blue-green algae) and tobacco chloroplasts. Biochim Biophys Acta 546:270–279

Oren A, Fattom A, Padan E, Tietz A (1985) Unsaturated fatty acid composition and biosynthesis in *Oscillatoria limnetica* and other cyanobacteria. Arch Microbiol 141:138–142

Oren A, Fischel U, Aizenshtat Z, Krein EB, Reed RH (1994) Osmotic adaptation of microbial communities in hypersaline mats. In: Stal LJ, Caumette P (eds) Microbial mats structure, development and environmental significance. Springer, Berlin, pp 125–130, 463 pp

Oren A, Kühl M, Karsten U (1995) An endevaporitic microbial mat within a gypsum crust: zonation of phototrophs, photopigments, and light penetration. Mar Ecol Prog Ser 128:151–159

Oren A, Sørensen KB, Canfield DE, Teske AP, Ionescu D, Lipski A, Altendorf K (2009) Microbial communities and processes within a hypersaline gypsum crust in a saltern evaporation pond (Eilat, Israel). Hydrobiologia 626:15–26

Ortí Cabo F, Pueyo Mur JJ, Truc G (1984) Las salinas marítimas de Santa Pola (Alicante, España). Breve introducción al studio de un medio natural controlado de sedimentación evaporítica somera. Rev Inv Geol 38(39):9–29

Padan E (1979a) Facultative anoxygenic photosynthesis in cyanobacteria. Annu Rev Plant Physiol 30:27–40

Padan E (1979b) Impact of facultatively anaerobic photoautotrophic metabolism on ecology of cyanobacteria (blue-green algae). In: Alexander M (ed) Advances in microbial ecology, vol 3. Plenum Publishing Corporation, New York, pp 1–48, 225 pp

Paerl HW, Pinckney JL, Steppe TF (2000) Cyanobacterial-bacterial mat consortia: examining the functional unit of microbial survival and growth in extreme environments. Environ Microbiol 2:11–26

Paerl HW, Steppe TF, Buchan KC, Potts M (2003) Hypersaline cyanobacterial mats as indicators of elevated tropical hurricane activity and associated climate change. Ambio 32:87–90

Pinckney JL, Paerl HW (1997) Anoxygenic photosynthesis and nitrogen fixation by a microbial mat in a Bahamian hypersaline lagoon. Appl Environ Microbiol 63:420–426

Post FJ (1977) The microbial ecology of the Great Salt Lake. Microb Ecol 3:143–165

Post FJ (1981) Microbiology of the Great Salt Lake north arm. Hydrobiologia 81:59–69

Potts M (1980) Blue-green algae (Cyanophyta) in marine coastal environments of the Sinai Peninsula; distribution, zonation, stratification and taxonomic diversity. Phycologia 19:60–73

Prášil O, Bina D, Medová H, Řeháková K, Zapomělová E, Veselá J, Oren A (2009) Emission spectroscopy and kinetic fluorimetry studies of the phototrophic microbial communities along the salinity gradient in the solar saltern evaporation ponds of Eilat, Israel. Aquat Microb Ecol 56:285–296

Rahaman AA, Ambikadevi M, Sosamma-Esso (1993) Biological management of Indian solar saltworks. In: Kakihana H, Hardy HR Jr, Hoshi T, Toyokura K (eds) Proceedings of the seventh symposium on salt, vol 1. Elsevier Science Publishers, Amsterdam, pp 633–643

Reed RH, Chudek JA, Foster R, Stewart WDP (1984a) Osmotic adjustment in cyanobacteria from hypersaline environments. Arch Microbiol 138:333–337

Reed RH, Richardson DL, Warr SRC, Stewart WDP (1984b) Carbohydrate accumulation and osmotic stress in cyanobacteria. J Gen Microbiol 130:1–4

Reed RH, Warr SRC, Richardson DL, Moore DJ, Stewart WDP (1985) Multiphasic osmotic adjustment in a euryhaline cyanobacterium. FEMS Microbiol Lett 28:225–229

Reed RH, Warr SRC, Kerby NW, Stewart WDP (1986) Osmotic shock-induced release of low molecular weight metabolites from free-living and immobilised cyanobacteria. Enzyme Microb Technol 8:101–104

Revsbech NP, Jørgensen BB, Blackburn TH, Cohen Y (1985) Microelectrode studies of photosynthesis and O_2, H_2S, and pH profiles of a microbial mat. Limnol Oceanogr 28:1062–1074

Richardson DL, Reed RH, Stewart WDP (1983) *Synechocystis* PCC6803: a euryhaline cyanobacterium. FEMS Microbiol Lett 18:99–102

Ritter D, Yopp JH (1993) Plasma membrane lipid composition of the halophilic cyanobacterium *Aphanothece halophytica*. Arch Microbiol 159:435–439

Roney HC, Booth GM, Cox PA (2009) Competitive exclusion of cyanobacterial species in the Great Salt Lake. Extremophiles 13:355–361

Rontani J-F, Volkman JK (2005) Lipid characterization of coastal hypersaline cyanobacterial mats from the Camargue (France). Org Geochem 36:251–272

Rothschild LJ, Giver LJ, White MR, Mancinelli RL (1994) Metabolic activity of microorganisms in evaporites. J Phycol 30:431–438

Roussomoustakaki M, Anagnostidis K (1991) *Cyanothece halobia*, a new planktic chroococcalean cyanophyta from hellenic heliothermal saltworks. Arch Hydrobiol 64:71–95

Roux JM (1996) Production of polysaccharide slime by microbial mats in the hypersaline environment of a Western Australian solar saltfield. Int J Salt Lake Res 5:103–130

Rushforth SR, Felix EA (1982) Biotic adjustments to changing salinities in the Great Salt Lake, Utah, USA. Microb Ecol 8:157–161

Sammy N (1983) Biological systems in north-western Australian solar saltfields. In: Sixth international symposium on salt, vol 1. The Salt Institute, Alexandria, pp 207–215

Schneider J, Herrmann AG (1980) Saltworks – natural laboratories for microbiological and geochemical investigations during the evaporation of seawater. In: Fifth international symposium on salt. Northern Ohio Geological Society, Cleveland, pp 371–381

Schoor A, Hagemann M, Erdmann N (1996) Non-radiometric assay for glucosylglycerol-synthesizing enzymes involved in the cyanobacterial salt adaptation. J Microbiol Method 27:139–145

Schoor A, Hagemann M, Erdmann N (1999) Glucosylglycerol-phosphate synthase: target for ion-mediated regulation of osmolyte synthesis in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Arch Microbiol 171:101–106

Seckbach J, Oren A (2007) Oxygenic photosynthetic microorganisms in extreme environments. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments. Springer, Dordrecht, pp 2–25, 811 pp

Shabana EF, Ali GH (1999) Phytoplankton activities in hypersaline, anoxic conditions II Photosynthetic activity of some sulphide-adapted cyanobacterial strains isolated from Solar Lake, Taba, Egypt. Water Sci Technol 40(7):127–132

Shahak Y, Arieli B, Binder B, Padan E (1987) Sulfide-dependent photosynthetic electron flow to proton translocation in thylakoids of the cyanobacterium *Oscillatoria limnetica*. Arch Biochem Biophys 259:605–615

Siegel BZ, McMurty G, Siegel SM, Chen J, LaRock P (1979) Life in the calcium chloride environment of Don Juan Pond, Antarctica. Nature 280:828–829

Siegesmund MA, Johansen JR, Karsten U, Friedl T (2008) *Coleofasciculus* gen. nov. (Cyanobacteria): morphological and molecular criteria for revision of the genus *Microcoleus* Gomont. J Phycol 44:1572–1585

Simon RD (1981) Gliding motility in *Aphanothece halophytica*: analysis of wall proteins in *mot* mutants. J Bacteriol 148:315–321

Sørensen KB, Canfield DE, Oren A (2004) Salt responses of benthic microbial communities in a solar saltern (Eilat, Israel). Appl Environ Microbiol 70:1608–1616

Sørensen K, Řeháková K, Zapomělová E, Oren A (2009) Distribution of benthic phototrophs, sulfate reducers, and methanogens in two adjacent salt ponds in Eilat, Israel. Aquat Microb Ecol 56:275–284

Steppe TF, Olson JB, Paerl HW, Litaker RW, Belnap J (1996) Consortial N_2 fixation: a strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. FEMS Microbiol Ecol 21:149–156

Stolz JF (1990) Distribution of phototrophic microbes in the flat laminated microbial mat at Laguna Figueroa, Baja California, Mexico. Biosystems 23:345–357

Sudo H, Burgess JG, Takemasa H, Nakamura N, Matsunaga T (1995) Sulfated exopolysaccharide production by the halophilic cyanobacterium *Aphanocapsa halophytica*. Curr Microbiol 30:219–222

Sugino M, Hibino T, Tanaka Y, Nii N, Takabe T, Takabe T (1999) Overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytica* acquires resistance to salt stress in transgenic tobacco plants. Plant Sci 137:81–88

Taher AG, Abd el Wahab S, Philip G, Krumbein WE, Wali AM (1995) Evaporitic sedimentation and microbial mats in a salina system (Port Fouad, Egypt). Int J Salt Lake Res 4:95–116

Takabe T, Incharoensakdi A, Arakawa K, Yokota S (1988) CO_2 fixation rate and RuBisCO content increase in the halotolerant cyanobacterium, *Aphanothece halophytica*, grown in high salinities. Plant Physiol 88:1120–1124

Takabe T, Uchida F, Shinagawa F, Terada Y, Kajita H, Tanaka Y, Takabe T, Hayashi T, Kawai T, Takabe T (2008) Overexpression of DnaK from a halotolerant cyanobacterium *Aphanothece halophytica* enhances growth rate as well as abiotic stress tolerance of poplar plants. Plant Growth Regul 56:265–273

Tel-Or E, Spath S, Packer L, Mehlhorn RJ (1986) Carbon-13 NMR studies of salt shock induced carbohydrate turnover in the marine cyanobacterium *Agmenellum quadruplicatum*. Plant Physiol 82:646–652

Thomas J-C (1984) Formations benthiques à Cyanobactéries des salins de Santa Pola (Espagne): composition spécifique, morphologie et caractéristiques biologiques des principaux peuplements. Rev Inv Geol 38(39):139–158

Tindall DR, Yopp JH, Miller DM, Schmid WE (1978) Physico-chemical parameters governing the growth of *Aphanothece halophytica* (Chroococcales) in hypersaline media. Phycologia 17:179–185

Uchida A, Hibino T, Shimada T, Saigusa M, Takabe T, Araki E, Kajita H, Takabe T (2008) Overexpression of DnaK chaperone from a halotolerant cyanobacterium *Aphanothece halophytica* increases seed yield in rice and tobacco. Plant Biotechnol 25:141–150

van Rijn J, Cohen Y (1983) Ecophysiology of the cyanobacterium *Dactylococcopsis salina*: effect of light intensity, sulphide and temperature. J Gen Microbiol 129:1849–1856

Volcani, BE (1944) The microorganisms of the Dead Sea. In: Papers collected to commemorate the 70th anniversary of Dr. Chaim Weizmann. Collective volume. Daniel Sieff Research Institute, Rehovoth, pp 71–85

Volkmann M, Gorbushina AA, Kedar L, Oren A (2006) The structure of euhalothece-362, a novel red-shifted mycosporine-like amino acid, from a halophilic cyanobacterium (*Euhalothece* sp.). FEMS Microbiol Lett 258:50–54

Waditee R, Incharoensakdi A (2001) Purification and kinetic properties of betaine-homocysteine methyltransferase from *Aphanothece halophytica*. Curr Microbiol 43:107–111

Waditee R, Hibino T, Tanaka Y, Nakamura T, Incharoensakdi A, Takabe T (2001) Halotolerant cyanobacterium *Aphanothece halophytica* contains an Na^+/H^+ antiporter, homologous to eukaryotic ones, with novel ion specificity affected by C-terminal tail. J Biol Chem 276:36931–36938

Waditee R, Tanaka Y, Aoki K, Hibino T, Jikuya H, Tanano J, Takabe T, Takabe T (2003) Isolation and functional characterization of *N*-methyltransferases that catalyze betaine synthesis from glycine in a halotolerant photosynthetic organism *Aphanothece halophytica*. J Biol Chem 278:4932–4942

Waditee R, Bhuiyan NH, Hirata E, Hibino T, Tanaka Y, Shikata M, Takabe T (2007) Metabolic engineering for betaine accumulation in microbes and plants. J Biol Chem 282:34185–34193

Walsby AE, van Rijn J, Cohen Y (1983) The biology of a new gas-vacuolate cyanobacterium, *Dactylococcopsis salina* sp. nov., in Solar Lake. Proc R Soc Lond B 217:417–447

Warr SRC, Reed RH, Stewart WDP (1984) Osmotic adjustment of cyanobacteria: the effects of NaCl, KCl, sucrose and glycine betaine on glutamine synthetase activity in a marine and a halotolerant strain. J Gen Microbiol 130:2169–2175

Warr SRC, Reed RH, Chudek JA, Foster R, Stewart WDP (1985) Osmotic adjustment in *Spirulina platensis*. Planta 163:424–429

Wharton RA Jr, Parker BC, Simmons GM Jr (1983) Distribution, species composition and morphology of algal mats in Antarctic dry valley lakes. Phycologia 22:355–365

Whitehead PR, Brown NL (1982) *Aha*III: a restriction endonuclease with a recognition sequence coding only A:T basepairs. FEBS Lett 143:296–300

Whitehead PR, Brown NL (1985) A simple and rapid method for screening bacteria for type II restriction endonucleases – enzymes in *Aphanothece halophytica*. Arch Microbiol 141:70–74

Wiangnon K, Raksajit W, Incharoensakdi A (2007) Presence of a Na^+-stimulated P-type ATPase in the plasma membrane of the alkaliphilic halotolerant cyanobacterium *Aphanothece halophytica*. FEMS Microbiol Lett 270:139–145

Wieland A, Kühl M (2000) Short-term temperature effects on oxygen and sulfide cycling in a hypersaline cyanobacterial mat (Solar Lake, Egypt). Mar Ecol Prog Ser 196:87–102

Wieland A, Kühl M (2006) Regulation of photosynthesis and oxygen consumption in a hypersaline cyanobacterial mat (Camargue, France) by irradiance, temperature and salinity. FEMS Microbiol Ecol 55:195–210

Woelfel J, Sørensen K, Warkentin M, Forster S, Oren A, Schumann R (2009) Oxygen evolution in a hypersaline crust: photosynthesis quantification by *in situ* microelectrode profiling and planar optodes in incubation chambers. Aquat Microb Ecol 56:263–273

Wright SW, Burton HR (1981) The biology of Antarctic saline lakes. Hydrobiologia 82:319–338

Wutipraditkul N, Waditee R, Incharoensakdi A, Hibino T, Tanaka Y, Nakamura T, Shikata M, Takabe T, Takabe Y (2005) Halotolerant cyanobacterium *Aphanothece halophytica* contains NapA-type Na^+/H^+ antiporters with novel ion specificity that are involved in salt tolerance at alkaline pH. Appl Environ Microbiol 71:4176–4184

Yannarell AC, Steppe TF, Paerl HW (2000) Genetic variance in the composition of two functional groups (diazotrophs and cyanobacteria) from a hypersaline microbial mat. Appl Environ Microbiol 72:1207–1217

Yopp JH, Tindall DR, Miller DM, Schmid WE (1978a) Isolation, purification and evidence for a halophilic nature of the blue-green alga *Aphanothece halophytica* Frémy (*Chroococcales*). Phycologia 17:172–178

Yopp JH, Miller DM, Tindall DR (1978b) Regulation of intracellular water potential in the halophilic blue-green alga *Aphanothece halophytica* (Chroococcales). In: Caplan SR, Ginzburg M (eds) Energetics and structure of halophilic microorganisms. Elsevier/North Holland, Amsterdam, pp 619–624, 672 pp

Yopp JH, Albright G, Miller DM (1979) Effects of antibiotics and ultraviolet radiation on the halophilic blue-green alga, *Aphanothece halophytica*. Bot Mar 22:267–272

Zavarzin GA, Gerasimenko LM, Zhilina TN (1993) Cyanobacterial communities in hypersaline lagoons of Lake Sivash. Mikrobiologiya (English translation) 62:645–652

Zheng W, Chen C, Cheng Q, Wang Y, Chu C (2006) Oral administration of exopolysaccharide from *Aphanothece halophytica* (Chroococcales) significantly inhibits influenza virus (H1N1)-induced pneumonia in mice. Int Immunopharmacol 6:1093–1099

Zvyagintseva IS, Gerasimenko LM, Kostrikina NA, Bulygina ES, Zavarzin GA (1995) Interaction of halobacteria and cyanobacteria in a halophilic cyanobacterial community. Mikrobiologiya (English translation) 64:252–258

Cyanobacteria, Oil – and Cyanofuel?

16

Roda Fahad Al-Thani and Malcolm Potts

Contents

R.F. Al-Thani (✉)
Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, POB 2713, Doha, Qatar
e-mail: ralthani@qu.edu.qa

M. Potts
Department of Biological and Environmental Sciences, College of Arts and Sciences, Qatar University, POB 2713, Doha, Qatar
e-mail: malcolm.p@qu.edu.qa

Summary

Global warming, the global carbon cycle, and current and future global trading in carbon credits (the carbon market), are beginning to dictate radical changes in human behaviour. In this context, the cyanobacteria figure prominently. Why? First, vast populations of ancient cyanobacteria and other microalgae are credited with the formation of Earth's oil deposits. Second, extant populations of cyanobacteria, most conspicuously marine picoplankton (Chaps 5 and 13) contribute significantly to the fixation of atmospheric carbon through their photosynthesis. Third, spills from the commercial trafficking of oil often accumulate in coastal regions where cyanobacterial mats are prevalent (Chap. 4), and this led to the examination of how these microorganisms participate in mitigation of the effects of oil pollution. Fourth, cyanobacteria may be a viable source of biofuel. As such, the rise of cyanobacteria, cyanobacteria and oil pollution, and cyanobacteria as a source of biofuel (cyanofuel) can be equated, respectfully, with Earth's past, present and future. In this chapter we emphasize connections between all three through consideration of cyanobacterial physiology, ecology and molecular biology. We wish to emphasize the persistence of cyanobacteria through geological time and their tenacious hold on carbon.

16.1 Background

In 1991 rapt attention of the world community focused on the Gulf War and the environmental consequences of oil pollution. Now, some two decades later, the potential after-effects of the prolonged spill of oil from a British Petroleum rig in the Gulf of Mexico during 2010 are the subject of considerable debate. In the case of the 1991 spill, within a comparatively short period the polluted coasts of the Gulf were found to support visually conspicuous growths of cyanobacteria, which raised interest in their role in oil degradation (Radwan and Al-Hasan 2000). In essence, 1991 provides the historical

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context for growing interest in the role of cyanobacteria with an emphasis on oil pollution. The review of Radwan and Al-Hasan provides the primary reference to the literature on the relationship between cyanobacteria and oil pollution. We take this as the background upon which the present appraisal, and new concepts, are developed.

16.2 What Is Oil?

The terms crude oil or, petroleum ("rock oil"), refer to natural deposits of hydrocarbons and other organic materials embedded within formations of porous rock on Earth. These flammable complex mixtures are characterized according to the types and proportion of hydrocarbons that they contain. With respect to the potential fate of these carbon compounds, it is informative to consider their contents (Speight 1999; Hyne 2001). The proportion of hydrocarbons in oil is highly variable and ranges from as much as 97% by weight in the lighter oils, to as little as 50% in the heavier oils and bitumens. The hydrocarbons in crude oil are mostly alkanes and cycloalkanes. Other organic compounds present contain nitrogen, oxygen and sulphur, and trace amounts of metals such as iron, nickel, copper and vanadium. The exact molecular composition of oil varies widely according to the particular deposit from which it comes; however the relative percentages of the chemical elements are constrained within fairly narrow limits: carbon (83–87%), hydrogen (10–14%), nitrogen (0.1–2%), oxygen (0.1–1.5%), sulphur (0.5–6%), metals (<1,000 ppm) (Speight 1999; Hyne 2001).

Four different classes of hydrocarbons are found in crude oil and, according to their relative percentages, they determine the physical and chemical properties of each oil: naphthenes (30% average, range 15–60%); paraffins (30% average, range 15–60%); aromatics (15% average, range 3–30%); asphaltics (6% average) (Speight 1999; Hyne 2001).

16.3 Origins of Oil

16.3.1 Biogenic

The theory of a biogenic origin for petroleum was developed in the 1930s following the identification of molecules in petroleum that have structural similarity with biomolecules (Kvenvolden 2006). It is accepted generally, though with some qualifications, that oil deposits are derived from the remains of vast ancient populations of zooplankton and algae (including cyanobacteria). For example, oil is present in the Upper Jurassic Arab A and Arab C carbonates of the Al Rayyan Field, Qatar, the upper reservoir of which contains stromatolites (Clark et al. 2004). However, the source, form, and distribution of oil-producing communities on the early Earth remains an enigma. Temperature has a critical affect on the process of oil formation. Below a certain minimum temperature the organic material in oil exists as kerogen, while above a maximum temperature the oil transforms to natural gas (Braun and Burnham 1993).

The finding of microorganisms within the subsurface of Earth's crust prompted a reappraisal and some debate on the source of biomarkers in petroleum and led to the suggestion, as promoted largely by the late Thomas Gold (1999), that thermophilic bacteria present there may be the source of a fraction of some oils. In contrast, gas-chromatographic analyses of 242 crude oils as well as 83 solvent extracts from upper to middle Paleozoic putative source rocks from the Timan-Pechora basin (Russia) identified n-alkanes and acyclic isoprenoids that were attributed to higher plants (waxes), cyanobacteria, microalgae and the green alga *Gloeocapsomorpha prisca* (Collister et al. 2004).

16.3.1.1 The Carbon Cycle

If one accepts a biogenic origin for oil, then oil exists because of the enzymatic fixation of atmospheric carbon dioxide (Chap. 17) and subsequent burial of the carbon in an aqueous environment. However, an appraisal of the extent to which this process occurred in marine versus brackish/freshwater settings, or even terrestrial settings is complicated by changing sea levels, shorelines and topography, and movements of oil since its formation. For example, the formation of surface tar sands in China is controlled by Alpine tectonics (Jiayu and Jianyi 1999). It would be useful to have this information in order to predict the likely forms of cyanobacteria that were involved in oil generation that is, their physiological potential and genetic make-up. The selection/manipulation of cyanobacterial strains for biofuel production, for example, is an important issue. Interestingly, organic-rich deposits within the Late Devonian Camrose Member of southern Alberta, Canada, are dominated by the "green" alga *Gloeocapsomorpha prisca*-derived alginite (Fowler et al. 2004). Microscopic analysis suggests that the organism (of enigmatic phylogeny) grew under high salinity conditions, which is consistent with the depositional environment of the Member. Given the present abundance and distribution of cyanobacteria in the world's oceans, it is not unreasonable to suggest that much oil derived from planktonic microorganisms that grew in open and coastal seas, much as unicellular cyanobacteria do today.

A new hypothesis proposes a critical role for carbon dioxide in the events that led to the formation of Earth's oxygen atmosphere (Dismukes et al. 2001). Through modelling of the chemistry of the Archean sea, it is proposed that bicarbonate was the thermodynamically preferred reductant before water in the evolution of oxygenic photosynthesis. A primitive bacterial ancestor to cyanobacteria utilized bicarbonate then, with the drop of carbon dioxide levels, cyanobacteria emerged using water instead of bicarbonate. Subsequently, it was

argued that the rise of cyanobacteria beginning at 2.8 Gya, and evolution of their CO_2-concentrating mechanism, coincides with the first evidence of atmospheric methane, the so-called "negative excursion" in the organic carbon isotope record (Schwartzman and Caldiera 2002). These two events were driven by declining carbon dioxide to oxygen ratios in the external environment, which accompanied the Archean greenhouse transition.

The true complexity of understanding global carbon budgets, and the role of cyanobacteria in the process of carbon fixation (Chap. 17), is evident from the finding by Bailey et al. (2008) that in the marine *Synechococcus* WH8102, there is significant alternative electron flow to O_2, a potential adaptation to the low iron environment in oligotrophic oceans. This alternative electron flow appears to extract electrons from the intersystem electron transport chain, prior to photosystem I. This finding makes it necessary to revise how one assesses the significance of optical measurements of photosynthetic pigments in the ocean and the way that ocean productivity is modelled. There is a further complication; the recent discovery of unicellular N_2-fixing cyanobacteria, widely distributed in tropical and sub-tropical seas, that lack genes for photosystem II, the site of O_2 generation (Zehr et al. 2008).

While terrestrial plants were the primary source of Earth's deposits of coal (but see Sect. 16.3.1), the role of terrestrial cyanobacteria and algae in either oil or coal formation is harder to evaluate. Cyanobacteria are present in many terrestrial environments, including extreme ones, and especially abundant in limestone regions. In the hot desert of the Kalahari of Botswana, for example, soil crusts are dominated by cyanobacteria that produce extracellular polymeric substances (EPS) that trap soil particles and decrease erosion. There are, however, few studies of the magnitude and controlling factors of soil CO_2 flux within these types of systems (Thomas et al. 2008). In contrast, in the cold desert of Victoria Land, Antarctica, form species *Nostoc commune* is dominant in mats and a model describing annual net C fixation gave estimates in the range of 14.5–21.0 g C fixed m^{-2} *Nostoc* mat (Novis et al. 2007). The relationship between thermal time and net annual C fixation by *N. commune* was shown to be linear. It appears, however, that it may take several years for colonies to achieve a size of around 50 mm.

16.3.1.2 Whitings

Cyanobacteria tend to be especially prevalent in regions where exposed limestone is abundant (Chap. 10). Diverse examples are found worldwide (Pentecost 2005). In these regions there is a complex interplay between the rock, atmosphere, precipitation/standing water and the cyanobacterial communities that grow on, and within, the limestone. Photosynthetic uptake of inorganic carbon can raise the pH adjacent to cyanobacterial cells, leading to precipitation of

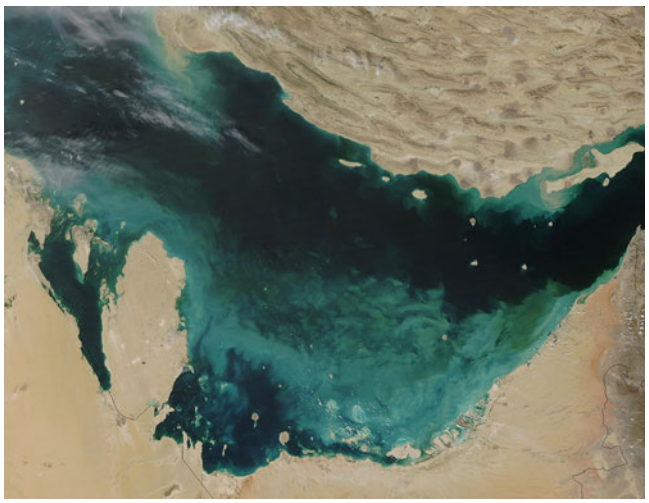

Fig. 16.1 A bloom (whiting) in the southern Arabian Gulf. The bloom colours the waters torquoise-blue in this true-colour Aqua Moderate Resolution Imaging Spectroradiometer (*MODIS*) image from 18 February 2003. The bloom surrounds the peninsula of Qatar, and stretches from Bahrain in the west, along the shores of the United Arab Emirates and Oman to the Strait of Hormuz (The image is owned by NASA with credit to Jeff Schmalz of the MODIS Rapid Response Team, and is made available to the public through Visible Earth at http://visibleearth.nasa.gov/view_rec.php?id=4983)

$CaCO_3$ (Riding 2006); a process that can be traced back at least 2.6 billion years (Altermann et al. 2006; Arp et al. 2001). Cyanobacteria belonging to the *Synechococcus* group are present in many marine and freshwaters. In these forms, it seems the nucleation sites for mineral precipitation are in the hexagonally-symmetrical protein S layer at the cell surface (Schultze-Lam et al. 1992; see Chap. 18). Laboratory investigation with *Synechococcus* PCC 8806 and PCC 8807 indicates that it is not cell density that determines precipitation, but rather, the elevation in pH that accompanies photosynthesis (Brady et al. 2004).

What is the significance of this relationship between limestone and cyanobacteria to the present account? Opinion among geologists is that the reduction of pCO_2 in the Proterozoic led to an enhanced capacity for calcification as well as the evolution of CO_2-concentrating mechanisms (CCMs) that actively transport HCO_3^- into the cells (Kah and Riding 2007; Lee et al. 2004, Chap. 17). A spectacular example of such cyanobacterial-mediated calcification is the appearance of "whitings" (Riding 2006). These milky waters, frequently visible from space (Fig. 16.1), occur typically in shallow coastal regions of the tropics and subtropics where rapidly-growing blooms of cyanobacteria enhance precipitation.

The direct correlation between in vivo sheath calcification of cyanobacteria, their carbon dioxide concentrating mechanisms (CCMs), and atmospheric CO_2 concentration, is an exceptionally powerful tool for interpreting prevailing atmospheric conditions over geological time. CCMs are induced in

extant forms of cyanobacteria under experimental conditions when pCO_2 is below ~0.36% (~10 times **p**resent **a**tmospheric **l**evel, PAL). The study of calcified filaments in stromatolites of the Society Cliffs area of Canada, imply pCO_2 levels of <0.36% at ca. 1,200 Ma. This inference is consistent with marine carbon isotope modelling that suggests pCO_2 of 7–10 PAL in the late Mesoproterozoic.

An informative review and chronology on whitings is provided by Lidz and Gibbons (2008). These authors propose that the persistence of "whitings" in the Arabian Gulf region may have led to the large deposits of oil there. In regard to the further significance of whitings, the capacity to grow cells in sufficient density, in expansive regions of coastal or inland waters, is of obvious relevance to the development of biofuel technologies and carbon sequestration.

16.3.1.3 Hydrocarbon Production by Cyanobacteria

Cyanobacteria produce diverse hydrocarbon secondary products (Jüttner 1991). The physiological function of many of these is unknown. *Calothrix* sp. PCC 7507 produces a range of sesquiterpenes including geosmin as well as the newly described compounds isodihydroagarofuran, eremophilone and 6,11-epoxyisodaucane (Höckelmann et al. 2009). Eremophilone was found mainly in the medium of axenic cultures and was shown to have acute toxicity to *Chironomus riparius* (insect) and *Thamnocephalus platyurus* (crustacean), but not for *Plectus cirrhatus* (nematode). A prodigious generation of hydrocarbons, principally ethylene gas, was documented for the Solar Lake, a coastal hot-brine environment in the Gulf of Aqaba/Eilat, where planktonic cyanobacteria and stromatolitic mats are abundant (Potts 1979).

16.3.2 Abiogenic Origin

An alternative to the concept that oil is a fossil fuel is the hypothesis that oil formed through abiogenic processes. One of the most vocal advocates of this was Thomas Gold (1999). He cited the finding of thermophilic bacteria in Earth's deep subsurface as a plausible explanation for the presence of biomarkers in extracted petroleum. While there are certainly examples of the generation of hydrocarbons through abiogenic processes, the consensus view of geochemists is that oil is derived from microorganisms (Glasby 2006; Collister et al. 2004).

16.4 Oil Spills – Natural and Man Made

There are many examples of the entry of oil and oil products into marine and fresh waters and this is a natural occurrence that must have occurred for millennia (Khan 2008). As such there has been continual pressure for natural selection of organisms with the capacity to degrade a range of different and complex hydrocarbons. One must question if cyanobacteria belong to this group.

A detailed summary of the major oil spills of the last century is available from The International Tanker Owners Pollution Federation Ltd, London (ITOPF 2009). The first major spills occurred during World War II (1939–1945), between January and June of 1942, when attacks by German U-boats on tankers off the east coast of the USA spilled 590,000 t oil. The first major commercial oil spill occurred on 18 March 1967, when the tanker Torrey Canyon ran aground on the Seven Stones Shoal off the coast of Cornwall, England. The tanker spilled 830,000 barrels (119,000 t) of Kuwaiti oil into the sea. On 25 January 1991, during the Gulf War, almost $1.5 \cdot 10^6$ t oil was dumped deliberately from Sea Island into the Arabian Gulf leading to an oil slick that covered some 1,500 km^2 of sea surface and blackened 480 km of coastline.

16.5 Role of Cyanobacteria in Oil Degradation and Reclamation

16.5.1 Resistance to Hydrocarbons

An initial reading of the literature leaves the impression that cyanobacteria generally have beneficial effects on the mitigation of oil spills, when in fact it seems clear to the authors that the intervention of cyanobacteria through physical and/or biochemical means is rather complex. For example, experiments with *Phormidium*- and *Oscillatoria*-dominated communities inhabiting a heavily polluted site in a coastal stream (Wadi Gaza, Palestine) demonstrated that both the cyanobacterial and eubacterial communities underwent marked shifts in phylogenetic composition concomitant with degradation of the petroleum compounds (Abed et al. 2002). In the eastern province of Saudi Arabia, including Abu Ali Island and the region around Jubail, there is a continuous input of oil from damaged oil wells, leaking pipes and discharge of ballast at loading terminals (Al Thukair et al. 2007). Analysis of the dominant non-heterocystous communities that cover oil-polluted sediments there, using Denaturing Gradient Gel Electrophoresis and 16S rRNA fingerprinting, indicated a drop in diversity moving from the lower to upper intertidal, largely as a consequence of the severity of increases in desiccation, salinity and temperature (Al Thukair et al. 2007). Paradoxically, these cyanobacterial communities are considered to be an obstacle to oil degradation since they build extensive mats that seal the surface, and encourage anoxic conditions that are not conducive to oil degradation (Barth 2003).

16.5.2 Roof Shingle

One cyanobacterial community that merits mention is one that produces conspicuous communities on the surface of an oil-product; asphalt (Chap. 18). Virtually every dwelling and commercial property in the United States with roof shingle supports an unusual and highly resilient microbial biofilm. The dominant microorganisms, according to geographic location are the cyanobacteria *Gloeocapsa cf sanguinea, Scytonema/Tolypothrix* sp., or the green alga *Neochloris* sp. Shingle itself is a malleable glass-fiber and asphalt (oil-derived) matrix with limestone filler, impregnated with rock granules at the surface, including solid zinc or copper-clad (redox active) granules that are intended to act as a biocide. Microbial cells (cyanobacteria, bacteria, fungi, yeasts, eukaryotic microalgae) infiltrate this matrix and develop a prominent and visually conspicuous biomass dominated by poorly characterized cyanobacteria (Chap. 18). Pigments of the cyanobacteria lend a red to black colouration to these growths. These are robust and physiologically-versatile immobilized aerophytic biofilms that are subjected to temperatures that exceed 100°C in summer months and that can fall below −30°C in winter, erratic cycles of desiccation and rehydration, intense UV irradiation, paucity of nutrients, atmospheric pollution including sulphur-rich acid rain, heavy metals including redox active Cu, and hydrocarbons that leach from the asphalt oil matrix. Most significantly, these stresses are all superimposed at random in complex, seasonal and diurnal permutations that drive evolution of the communities over decades. The extreme environmental gradients that impose these stresses reach from the Great Lakes to Florida, from the East coast to the mid West, to the Pacific Northwest, and even to Hawaii. Our group I intron sequence analyses suggest a continuum of taxonomic diversity in these populations (D.R. Wright, R.F. Helm and M. Potts, unpublished data; Chap. 18, Fig. 18.8).

16.5.3 Degradation of Hydrocarbons

16.5.3.1 Consortia

All available evidence from the rock record is that photoautotrophy has served as the foundation of the world's ecosystem since at least 3,500 Ma ago (Chap. 2) and, today, the genus *Prochlorococcus* genus is the most abundant phototroph on Earth (Chap. 20). Although photoautotrophy appears to be the normal mode of growth of cyanobacteria, all forms have the potential for photolithotrophy and there are also examples of cyanobacteria that are photoheterotrophic (see Muhling et al. 2005 for refs). Because populations of cyanobacteria persist in coastal regions, where oil spills tend to accumulate, researchers questioned: can cyanobacteria degrade actively, that is enzymatically, oil-based hydrocarbons?

A critical review by Van Hamme et al. (2003) evaluates how whole cells, enzymes and genetic engineering of eubacterial strains of *Rhodococcus, Pseudomonas, Mycobacterium, Sphingomonas, Burholderia, Azoarcus* and others, are being used in enhanced oil recovery, de-emulsification, desulphurization, denitrogenation, upgrading of petroleum fractions and biosensors. A considerable amount of information is available on how they do so. Breakdown involves a complex interplay of physical and chemical processes, as well as the intervention of diverse genomes. It is germane to point out the analogy of the abiogenic and biogenic aspects of oil formation (see above) and dissolution, in a chronological context, respectfully. Collectively, these antagonistic processes must have occurred since the formation of oil on Earth; becoming exacerbated, from an ecological perspective, since the first major oil spill of a tanker in modern times. However, one must question the intervention of the cyanobacteria over geological time in these aspects of petroleum dynamics.

The breakdown of oil is as complex as is oil diagenesis. For example, it is stated widely that cyanobacteria provide oil-degrading bacteria with the necessary oxygen, microhabitat, nitrogen and organics to sustain hydrocarbon heterotrophy (Abed et al. 2009; Sanchez et al. 2005). In a heterotrophic biofilm O_2 transfer by diffusion is limited by approximately 20 nmol cm^{-2} min^{-1} (Roeselers et al. 2008). However, the areal net oxygen production in an active phototrophic biofilm, at a photon flux density of 1,000 μmol photon m^{-2} s^{-1}, is approximately a factor of two higher. Therefore oxygen produced by phototrophs could, potentially, fulfill a substantial proportion of the demand of the heterotrophic bacteria. One can appreciate how cyanobacteria would contribute, essentially "passively," to oil degradation. From a review of the literature through 2000, from a decade of studies since the Gulf War, Radwan and Al-Hassan (2000) concluded that there was no rigorous evidence for hydrocarbon oxidation by cyanobacteria due to the lack of studies with axenic cultures.

The range of investigations of bacterial consortia in the context of oil degradation is broad. A conspicuous depletion of hydrocarbons spilled by the tanker *Prestige*, along the Atlantic coast of Galicia (Spain), was attributed to consortia of heterotrophic bacteria, fungi and cyanobacteria (*Anabaena, Oscillatoria* and others). Curiously, highest depletion rates occurred only in regions of the coast where freshwater flowed through the contaminated shore rocks (Gallego et al. 2006). Enrichment cultures using petroleum as the sole carbon source did lead to the appearance of cyanobacteria. The most abundant phototrophs in a consortium growing in waste waters from an oil refinery and nitrogen plant were *Phormidium foveolarum,* diatoms and algae (Antic et al. 2006). Despite the affinity of cyanobacteria for the oil substrate in *Microcoleus* consortia, changes in oil composition were found to be small (Oteyza et al. 2004). With the goal of

Fig. 16.2 Northernmost extremity of Qatar close to the town of Madinat Ashamal. Layered beachrock consists of an upper white layer and a lower brown to black layer (*right* of figure). The *brown* colouration is attributed to epilithic and endolithic coccoid cyanobacteria. In the foreground pink and grey-blue coloured mats of *Schizothrix* sp. and other filamentous forms associate with sand and oil (see Fig. 16.3)

preparing trickling filters to treat oil-contaminated wastes, artificial microbial consortia were grown as biofilms on gravel particles (2–3 cm diameter) and glass plates. Despite slow colonization (with full development after 4 months) the biofilm showed a capacity for hydrocarbon removal, with phototrophic microorganisms (cyanobacteria, picoplankton, diatoms) as the primary colonizers and with *Acinetobacter calcoaceticus* and nocardioforms attached to the cyanobacterial filaments (Al-Awadhi et al. 2003). An essential prerequisite was the presence of KNO_3 as fertilizer. Precoating the gravel or glass surface with crude oil prevented development of the biofilm, which was attributed to the failure of the primary colonizers to attach to the hydrophobic surface.

Abed and Koster (2005) investigated the potential of ten strains of mat-forming cyanobacteria from saline environments to degrade n-octadecane, pristine, phenanthrene, and dibenzothiophene. These model compounds for petroleum constituents represent straight chain and branched alkanes, aromatic hydrocarbons, and organosulphur compounds. Hydrophobic clay was used as a carrier to improve accessibility to these hydrophobic substrates. Non-axenic strains of *Aphanothece halophytica, Dactylococcopsis salina, Halothece* sp. *Oscillatoria* sp. and *Synechocystsis* spp. were able to degrade n-alkanes. Again, it was concluded that the degradation was due to the associated heterotrophic eubacteria rather than cyanobacteria.

The presence of oil may in fact be detrimental to cyanobacteria. Euendolithic cyanobacterial populations dominated by species of *Hyella* and *Solentia* bore calcium carbonate substrates, including ooids, in coastal environments of the Arabian Gulf (Fig. 16.2). The numbers and distribution of these rock-boring cyanobacteria in ooids diminished significantly following oil spillages of the 1991 Gulf War. Presumably this reflects coating of the grains that may prevent further colonization and/or cause death of the extant population due to reduction of penetration of sunlight and gas exchange (Al-Thukair 2002).

More recently, samples of benthic cyanobacterial mats from Ebro Delta, Tarragona, Spain, were studied in a tubular reactor, packed with perlite soaked with Casablanca crude (Sanchez et al. 2006). The liquid water circulating through the reactor contained no carbonate or inorganic carbon source and was under a nitrogen atmosphere with only traces of oxygen. The microaerophilic conditions eliminated the possibility that cyanobacteria could grow at the expense of CO_2 generated through aerobic oxidation of hydrocarbons in the oil. Under these conditions no evidence was obtained for growth of the cyanobacteria, nor any capacity of the cyanobacteria for growth with oil as a carbon source. Further, in axenic culture, monospecific *Phormidium animale* collected from oil-polluted freshwater pond mats in East of Kalimantan (Borneo, Indonesia), did not exhibit any degradation of hydrocarbons in the range of C13-C35 carbon atoms either in autotrophic or heterotrophic growth (Chaillan et al. 2006). In another study, Cohen (2002) compared the responses of cyanobacterial mats from polluted sites along the African coasts of Suez to those growing in the pristine Solar Lake (Sinai). Both types of mat showed efficient degradation of crude oil in the light, followed by formation of intense growths of *Phormidium* and *Oscillatoria*. Oxygen microelectrodes detected marked inhibition of photosynthesis following exposure of mats to oil, and isolated axenic strains had no such capacity for degradation that was attributed to aerobic heterotrophs such as *Marinobacter* sp.

From a study of nutrient and hexadecane inputs and their effects on community composition and denitrification in river biofilms, correlation analysis demonstrated a significant relationship ($P < 0.05$) between denitrification rate and the

biomass of algae and heterotrophic bacteria but not with cyanobacteria. In fact cyanobacteria were a minor component of the biofilm under all experimental conditions tested. However addition of carbon/nitrogen/phosphorus did give a significant increase in cyanobacterial biomass, especially in the presence of hexadecane (Chenier et al. 2006). These findings may be attributed to how the cells were cultured; in this case on polycarbonate strips in rotating annular reactors.

Using a different approach some authors studied the effects of oil on pristine cyanobacterial mats. In one study, Lliros et al. (2008) followed the degradation of two crudes (Maya and Casablanca) on microbial mats subject to tidal movements in the Ebro Delta, Spain. Among filamentous cyanobacteria *Microcoleus chthonoplastes* was found to be the most resistant to the two oils, unlike other forms that tolerated Casablanca, but not Maya. Unicellular cyanobacteria seemed to be resistant to both oils with the exception of a *Gloeocapsa* morphotype that was sensitive to both. It is interesting to speculate on what features of the cyanobacterial morphotypes lend a sensitivity to one oil or another. Maya is a sulphur-rich heavy crude that is predominantly aromatic, whereas Casablanca is aliphatic with a low viscosity. The crude-oil additions had a significant effect on certain components of the heterotrophic community. For example, Casablanca induced an increase in numbers of anaerobic heterotrophs while Maya decreased their numbers.

16.5.3.2 Cultures

In what appears to be a definitive, yet singular study, Raghukumar et al. (2001) reported hydrocarbon degradation by pure cultures of cyanobacteria under defined conditions. Axenic cultures of *Oscillatoria salina* BDU 20631, *Plectonema terebrans* BDU 91211, *Aphanocapsa* sp. BDU 50261 (National Facility for Marine Cyanobacteria (NFMC), a publicly accessible culture collection centre at the Department of Biotechnology, Bharathidasan University, India) were grown photoautotrophically in artificial seawater medium. Seven-day cultures were used as inocula for fresh media containing 1% Bombay crude and, in separate experiments, with 0.1% pure hexadecane. Gravimetric and gas chromatographic analyses indicated that between 45% and 55% of Bombay crude, and 50–65% of pure hexadecane, were removed in the presence of live cyanobacterial cells within 10 days. Controls in these experiments included the use of heat-killed cells, calculation of adsorption of hydrocarbons to cells, monitoring of cultures microscopically as well as with nutrient medium to detect bacterial contamination.

A study by Kumar et al. (2009), which included a further summary of relevant literature, reported degradation of naphthalene and anthracene by axenic cultures of hypersaline strains of *Phormidium corium, Phormidium* sp., *P. augustissimum* and *P. tenue* from the Culture Collection Centre of Department of Microbiology, also at Bharathidasan University, India. In this case the level of hydrocarbons tolerated by the strains was an order of magnitude greater than that in the Raghukumar et al. (2001) study. However, there was no documentation of the criteria used to assess whether cultures were axenic.

16.5.3.3 Putative Cyanobacterial Genes for Hydrocarbon Degradation?

The demonstration by Raghukumar et al. (2001) of hydrocarbon degradation by axenic strains, under defined experimental conditions, raises the question of the genetic basis for this trait. In fact a thorough biochemical analysis (K_m, V_{max}) is warranted. What gene products are involved? Is there any degree of conservation between them and the gene products of eubacteria, for which considerable information is available? Would one expect to find such conservation? Many genome sequences of taxonomically-diverse cyanobacteria are available, and this makes it possible to attempt to answer these questions.

Operons (on both chromosomes and plasmids) involved in polycyclic aromatic hydrocarbon degradation in *Pseudomonas, Burkholderia, Nocardioides, Rhodococcus*, *Mycobacterium* and *Sphingomonas* contain some 76 individual genes (Van Hamme et al. 2003), many of which encode Rieske-type iron-sulphur proteins. An in-depth appraisal of whether there are potential orthologues of these gene products in cyanobacteria is beyond the scope of this chapter. However, we did perform a preliminary analysis that identified the following features: DoxA in *Pseudomonas* is a naphthalene dioxygenase in the Rieske-ferredoxin superfamily that showed significant (but low) sequence similarity to phosphoglucomutase and cytochrome b6-f complex (Reiske iron-sulphur) subunit proteins in 11 different cyanobacteria. Of particular interest, however, was the finding that NidA, the large subunit of a dioxygenase involved in pyrene and benzo (a)pyrene degradation in *Mycobacterium* sp. PYR-1 showed appreciable (up to 35% identity) sequence similarity to cytochrome b6-f complex (Rieske iron-sulphur) proteins in a range of cyanobacteria (data not shown).

DfdR is a GAF sensor protein in the actinomycete *Rhodococcus* sp. (Lida et al. 2009). The GAF acronym derives from the first three classes of proteins found to have the domain architecture i.e. (c**G**MP-binding PDEs, ***A****nabaena* adenylyl cyclases, and *Escherichia coli* **F**hlA (Aravind and Ponting 1997). It is proposed that DfdR identifies dibenzofuran and hydrocarbon aromatic compounds within the cell via the GAF-like domain of the protein and then activates transcription by binding to the promoter of *dfdA1* (Lida et al. 2009). Database searches by these authors revealed some 51 proteins with both GAF and helix-turn-helix domain architecture in a range of bacteria, including cyanobacteria (for example AAA50358 of *Anabaena* PCC 7120). All of these are functionally cryptic proteins.

Our own database searches identified some 56 sequences in a range of taxonomically-diverse cyanobacteria with very high sequence similarity (E values of 2e-14 to 2e-28) to AlkN, the putative methyl-accepting hydrocarbon chemosensor. However, given the overall conservation of enzymatically diverse proteins in the HAMP superfamily (**H**istidine kinases, **A**denyl cyclases, **M**ethyl-accepting proteins and **P**hosphatases) to which AlkN belongs and that include, for example, regulators of phototaxis in cyanobacteria, little can be drawn from our particular analysis. However, the concept that cyanobacteria may have chemotactic attraction to oil does seem worth investigation.

Curiously, hexadecane treatment (at 0.2%) of *Anabaena* PCC 7120 resulted in a twofold increase in mRNA and fourfold increase in levels of a cytochrome P450 encoded by a gene (*cyp110*) present on the *nifD* excision element (Torres et al. 2005). The treatment resulted in the appearance of a Cyp110-immunoreactive protein that co-migrated with purified Cyp110 in electrophoretic analysis. In this careful study it was noted that Cyp110 was only detected in hexadecane-treated cultures and was localized in the membrane fraction. However, hexane, octane and dodecane were all toxic to *Anabaena* PCC 7120 and the available data led the authors to suggest that Cyp110 does not participate in alkane degradation rather, it binds long-chain polyunsaturated fatty acids.

Among contemporary populations of cyanobacteria, the marine unicellular forms *Prochlorococcus* and *Synechococcus* (picocyanobacteria; Chap. 20) are the smallest and, numerically, dominate the phytoplankton of tropical and subtropical oceans (but not polar oceans; Chap. 13), where they are responsible for a significant fraction of global photosynthesis (Rocap et al. 2003). These populations are susceptible to oil spills, full annotated genome sequences are available, and therefore their subsequent responses to oil are of some interest. Picocyanobacteria sampled from waters of the Arabian Gulf grew well in inorganic media supplemented with 0.1% crude oil and cells survived concentrations of up to 1% (Al-Hasan et al. 2001). It was reported, following studies with electron microscopy, that these cells accumulated hydrocarbons (aliphatic n-hexadecane, aromatic phenanthrene) and crude oil intracellularly, within the interthylakoid space. Such uptake of alkanes in Gram negative bacteria, which have a comparable cell wall structure to cyanobacteria (but see Chap. 18 for qualifications), is thought to be mediated by the outer membrane protein AlkL; a product of the *alkBFGHJKL* operon in *Pseudomonas putida* (Van Hamme et al. 2003). When we used the amino acid sequence of *Pseudomonas* AlkL in BLAST searches against cyanobacterial sequences in the available public databases, 11 were identified with significant scores, but with no greater than 28% sequence identity to AlkL.

In the study of Al-Hasan et al. (2001) described above, despite the apparent uptake of oil by cells, no evidence was obtained for hydrocarbon oxidation in cultures using gas liquid chromatographic analysis. If oil uptake is indeed a property of cyanobacteria growing *in situ* (e.g. Figs. 16.3 and 16.4), in oil polluted waters, what are the effects of such uptake on vital features of cells such as changes in their buoyancy (that may influence distribution in the water column – see Chap. 18 – and possibly predation), and how do cell surface changes (Chap. 18) influence any uptake? In regard to buoyancy regulation, it can be noted that this property is not described for picocyanobacteria (Stockner et al. 2000). Also would not extracellular sheaths and mucilages, which are a common feature of cyanobacteria, act as a barrier to the uptake of oil? Such extracellular polysaccharides are especially prominent in those forms of cyanobacteria which form mats where oil tends to accumulate. One strain of cyanobacterium, J1, excretes an emulsifying agent that can sequester oil through formation of emulsions of hydrocarbons and oils in liquids such as water. In this case the emulsifying agent is a high molecular mass extracellular polysaccharide (Shilo and Ali 1987).

Fig. 16.3 Close-up view of mats in Fig. 16.2. Brown coloured beachrock is in the *upper* part of the figure. Grey areas are a mixture of pink *Schizothrix* mat, oil and sediment

Fig. 16.4 Diversity and complexity of oil/cyanobacteria communities at Madinat Ashamal. For scale; the conical gastropod shells are 1–2 cm long. Black areas are a mixture of predominantly crude oil, with sediment and cyanobacterial mats. Lighter areas are mats with lesser amounts of oil and or with a covering of sand. Colonies of coccoid cyanobacteria give a pustullate texture to the surface of some regions of the communities

Given the close proximity of heterotrophic oil-degrading bacteria and cyanobacteria in mixed consortia, as evaluated here, it is important to question the potential for genetic exchange between these different groups. For example, the bacterium *Polaromonas* JS666 has physiological versatility toward hydrocarbon substrates including the ability to grow on *cis*-1,2-dichlorethene as the sole carbon source (Mattes et al. 2008). The bacterium contains a number of mobile genetic elements, and many genes involved in hydrocarbon degradation, some of which are carried on plasmids. Interestingly, it carries the cyanobacterial miniature inverted-repeat transposable element (MITE) referred to as "Nezha," which is thought to have been acquired from cyanobacteria through recent horizontal gene transfer, as well as polyketide synthase gene clusters most related to cyanobacterial toxin gene clusters (Mattes et al. 2008).

16.5.3.4 Other Studies at the Molecular Level

Two additional studies are considered here to emphasis the conflicting data relating to oil degradation by cyanobacterial populations, in this case *in situ*. In a study of oil polluted waters in Toyama Bay, Japan, denaturing gradient gel electrophoresis (DGGE) of the amplified V3 region of 16S rDNA identified bands that corresponded to cyanobacteria but only in samples where oil was present (Tanaka et al. 2008). Yet, in a study of a simulated oil spill using mangrove sediments of Todos os Santos bay on the northeast coast of Brazil and using quantitative PCR for *rrs* and *nifH* genes, and *nifH* clone library sequencing, no sequences related to cyanobacteria were observed in any of the libraries (Taketani et al. 2009), despite the fact that cyanobacteria are often prominent in oil-associated environments and play a major role in nitrogen fixation in mangrove environments (Potts 1984).

Perhaps to most, the term "oil degradation" conjures immediately the concept of enzymatic dissolution and removal. In fact, with respect to cyanobacteria, one must see their potential role as rather more cryptic.

16.5.3.5 Physical Factors

The complexity of oil degradation can be illustrated through consideration of the careful study of Minas and Gunkel (1995). Using Bunker C, the major oil pollutant of the North Sea, and with the assumption that biodegradation follows Michaelis-Menten type kinetics, they measured V_{max} and K_M at 4°C and 18°C (to model winter and summer conditions, respectively). At both temperatures K_M for degradation was about 40 ppm, whereas V_{max} was 3–4 times higher at 18°C (summer); not surprising perhaps. However, what seems rather counterintuitive, was their conclusion that abiotic factors such as erosion and dispersion, rather than degradation, were responsible for enhanced oil removal, particular during winter. For example, high rates of degradation during winter were attributed to frequent storms, and turbulence, which resulted in greater dispersion of oil. At this point, these findings should be placed in context with regard to sub- and tropical environments where cyanobacteria are the prevalent plankton.

High speed, in-line digital holographic cinematography has been used to study the effects of mixing the dispersant COREXIT 9527 with crude oil (Gopalan and Katz 2008). It was found that after the oil droplet fragments, there remains an elongated tail, rather like the shape of a comet, as a consequence of the low local surface tension enhanced by the dispersant. At high dispersant to oil ratios, these thin tails extend from the oil droplets, and are stretched by the flow (turbulence). Break-up of these thin threads produces very small oil droplets, which is the desired effect during cleanup of an oil spill.

These examples serve to illustrate how nano features, superimposed with prevailing conditions of temperature, irradiation, salinity and evaporative loss (all features that cyanobacteria deal with most effectively) must be considered to have formative roles in driving the interaction between petroleum and a cyanobacterial cell. Because cyanobacteria obviously come into close physical contact with oil, one must question the role of cell surface properties in the interaction; as alluded to earlier in this discussion. Using a biphasic water-hydrocarbon test system, it was shown that all benthic cyanobacteria tested had a hydrophobic cell surface character, while all planktonic forms tested were hydrophilic (Fattom and Shilo 1984). Mechanical shearing of the cell surface, as well as chemical removal of the cell wall, demonstrated that the hydrophobicity was confined to the outer surface layers. Now, one must question how a cell of a planktonic *Synechococcus*, represented as a prolate ellipsoid and "dispersed" in a rotating Stokes (Einstein) flow (Seddon and Mullin 2007) i.e. in an oil suspension, with an hydrophilic cell surface, could influence oil dispersal. Equally one must extrapolate to, and understand, the biophysical contrasts in a population of an immobilized biofilm of cyanobacteria (mat) challenged with the deposition of a veneer of crude oil. These are difficult questions to answer and ones for which there are few data. It seems the interactions of benthic and planktonic cyanobacteria with oil may be rather different.

16.6 Cyanobacteria and the World Carbon Economy

16.6.1 Carbon Capture and Storage (CCS): Potential Problems

Carbon capture and storage (CCS) means the contrived means by which CO_2 is either removed or diverted from emission sources, and delivered to, and stored in, the ocean, terrestrial environments (vegetation, soils, and sediments),

or geologic formations such as depleted oil and gas fields (Beecy and Kuuskraa 2001; Sundquist et al. 2008; Azar et al. 2006). One example is where methane gas is produced from offshore gas fields, then transferred ashore by pipeline. Using existing oil-refinery technology, methane is then converted into H_2 and CO_2. The CO_2 is separated using membrane technology, and returned offshore, and stored in the oilfield, perhaps several km below sea level, instead of being vented into the atmosphere from a power station (UNEP 2006). Another suggestion is that CO_2 may be injected directly into the oceans. The CO_2 could be injected via a pipeline or from a moving ship. Alternatively, it could be deposited onto the deep seafloor at depths below 3,000 m, where CO_2 is denser than water (UNEP 2006). These technologies, however, are still in the research phase; CCS is a novel concept, largely untested with respect to its geological dimension and it requires evaluation and monitoring over time in the same way that the storage of nuclear waste will.

The great concern is the potential negative impact these CCS activities may have on the ocean environment. For example, the creation of CCS traps will be implemented in regions where oil excavation has been vigorous, such as in depleted oil and gas fields. In some cases, this means in coastal waters where cyanobacteria are often encountered. Of concern is the leakage of CO_2 from these reservoir traps, either through physical (perhaps tectonic related) means, or even through terrorist activities. Marked stimulation of cyanobacterial blooms would have environmental consequences such as fish kills and the potential for growth of toxin-producing forms.

16.6.2 Carbon Sequestration and Cyanofuel

In contrast to CCS, carbon sequestration refers to deliberate trapping of CO_2 in biomass through photosynthesis. Here, we arrive at the concept of cyanofuel. Our energy needs, whether they are for travel, food, shelter or other human activities, are as intractable as they are intricately linked. And, as we face and struggle with these energy needs, vanishing resources, the crisis of sustainable development, and world poverty, it is a matter of some debate whether the twentieth century will represent continued progress, or whether a long, steady, and debilitating decline will, or has, set in (National Geographic Special Issue 2009).

Rhetoric over the use of cyanobacteria and other microalgae as biofuels is rampant, as even a cursory search of the internet using the term "biofuel" indicates. The reality, however, is a paucity, and superficiality, of information on the contrived, and controlled, growth of microalgae at the scale needed to make this proposition feasible economically (NAS 2009). Yet claims, predictions and calculations of production costs, potential yields, estimates of sizes of facilities needed are awash in the popular media. In contrast, there are numerous peer-reviewed academic publications about the growth and physiology of cyanobacteria enclosed in small-scale stirred reactors that no doubt led to the notion that simple scale-up would be the way to go (Benemann 2008). Scientific opinion, however, is that a sustainable cyanofuel industry must rely on open-air cultivation of cyanobacteria and, even then, that technology is a long, long way from commercial maturity (Beneman 2002, 2008; Benemann and Oswald 1996; Benemann et al. 1982; Sheehan et al. 1998; Cadoret and Bernard 2008). Ironically, after some 30 years of competitive basic research in the physiology and molecular biology of cyanobacteria, it appears that everyone is now "on board" for cyanofuel discovery.

In terms of what is known about cultivation of cyanobacteria, Cyanotech Corporation (http://www.cyanotech.com/) was established 25 years ago in Hawaii and was the first company growing microalgae to obtain ISO 9001:2000 certification. Other major producers of *Arthrospira* ("Spirulina") outdoors include Earthrise Farms, in California, and Sosa Texcoco, Mexico (Belay 1997). Although *Arthrospira* is not an efficient oil producer, the concepts used in its cultivation are seminal, but still limiting when considering production costs and the biomass needed to have a viable global cyanofuel industry (Chap. 26).

There are other issues to consider. The regulatory aspects of carbon trading may be hard to accommodate. For example, the use of cyanobacteria in trapping of carbon dioxide from smoke stacks of power plants, while technologically sound, will likely falter in the long term due to the opinion of regulatory bodies that such fixed carbon is old, rather than new, fixed carbon. At the Meeting of Commercial Aviation Alternative Fuels Initiative (CAAFI) in Washington, DC (30 Sep -to 2 Oct 2009) it became clear that a major driver of the biofuel industry is the United States Air Force. As such, progress in the development of cyanofuel, promoted through bodies such as the Defense Advanced Research Programs Agency (DARPA) may remain cryptic.

In the past, the U.S. Department of Energy has invested some effort in studying the potential and the drawbacks in the development of biofuel (Benemann 2008). Most recently (November, 2009), at the Pacific Rim Summit on Industrial Biotechnology and Bioenergy in Honolulu, Hawaii, the DOE revealed its intentions to further pursue advanced algae-based biofuels that can be drop-in replacements for diesel and gasoline. Another agency, the National Renewable Energy Laboratory (NREL), is involved in an ambitious range of biofuel projects in 2009 and 2010, including establishment of a bioenergy-focused microalgae strain collection using high throughput methods, development of algal-based jet fuel, and collaboration with Israel and other US partners in the development of cost-effective algal biofuel (NREL 2009).

A number of patents deal with the development of methods to improve yields of biofuel. For example, Nobles and Brown (2008) refer to strains of *Synechococcus* sp. PCC 7002, *Synechococcus leopoliensis* strain UTCC100, *Agmenellum quadruplicatum* UTEX B2268, and *Synechococcus* ATCC 27264 engineered with a portion of a bacterial operon that leads to synthesis of cellulose. Cyanobacteria producing dry weight yields of 81.9% neutral lipids and that can be induced to lyse in the presence of Ni^{2+} are disclosed by Liu and Curtis (2009).

A speculation is proposed whereby a cyanobacterium is endowed with the capacity for heterologous fermentative metabolism – the "Photanol" concept (Angermayr et al. 2009); the proposal being the channeling of Calvin cycle metabolites into fermentative pathways for the production of alcohols and hydrogen (Hellingwerf et al. 2009).

In spite of all of these innovative methods and speculations being developed to engineer strains (from culture collections), to improve yields, to improve lysis efficiency, and otherwise, one must bring an understanding of cyanobacterial ecology to the fore and question the robustness of these chimerical strains, and ask how they would (will?) fare under the demanding environmental conditions of those regions where outdoor growth must be pursued.

16.7 The *Azolla* Event

Azolla is a free-floating widespread genus of freshwater fern that enters into symbiotic association with the filamentous cyanobacterium *Anabaena azollae*. The latter provides *Azolla* with a source of fixed atmospheric nitrogen that sustains growth of the fern (Chap. 23). Today, *Azolla* is used extensively as a "green manure" in rice cultivation. Compelling evidence for the added importance of this symbiosis through geological time came from the Arctic Coring Expedition (ACEX) 302 of the Integrated Ocean Drilling Program (IODP). In this programme, unique drill cores were obtained from the Lomonosov Ridge (and adjacent areas) in the central Arctic (Backman et al. 2006; Brinkhuis et al. 2006). Preliminary analyses of the laminated sediments suggested that a vast biomass of *Azolla* grew and accumulated in the then isolated and stratified Arctic Ocean during the mid Eocene (~48.5 Ma), for a period of at least 8×10^5 years. Conditions of warm temperature, low salinity, insolation, lack of mixing with anoxic bottom waters (Stein 2006), can be considered conducive to formation of photosynthetic populations and subsequent formation of oil. In fact, the U.S. Geological Survey (USGS) estimates there are 33 geologic provinces north of the Arctic Circle that are prospective for petroleum. *"The sum of the mean estimates for each province indicates that 90 billion barrels of oil, 1,669 trillion cubic feet of natural gas, and 44 billion barrels of natural gas liquids may remain to be found in the Arctic"* (84% is expected to occur in offshore areas: Bird et al. 2008). Perhaps with some optimism, several oil companies such as TOTAL Lubricants USA, Inc. now manufacture a range of hydraulic lubricants with the AZOLLA brand name.

16.8 Future Perspectives

World attention was drawn to the recent oil spill in the Gulf of Mexico, which justifies some comment here. As of writing this coda, the oil issuing from the bore of the British Petroleum drilling rig that sank on April 11th, 2010, approximately 50 miles from the Lousiana coast, USA, appears to be staunched after some 90 days of flow. The political, economic and social hiatus, as well as the ongoing acrimonious debate caused by this spill contribute much uncertainty over the actual amount, distribution and fate, of the spilled oil. It seems a proportion was recovered, while the remainder either evaporated or was dispersed physically and chemically into gulf waters in the form of minute droplets, floated passively around the gulf as aggregates of tar and surface slicks, was deposited ashore, or is buried in sand and ocean sediment. The long-term ecological consequences of this spill are hard to gauge. In the context of the present review it seems essential that previous studies on the importance of cyanobacteria in different environments of the Gulf of Mexico such as in sub-tropical estuaries (Murrell and Lores 2004), and their complex interactions, for example with podoviruses, in open gulf waters (Huang et al. 2010), be used as examples of benchmarks (controls) for future monitoring studies. The studies on coastal mat communities that were initiated following the 1991 Gulf War spills (as reviewed here and initially by Radwan and Al-Hasan 2000) also provide a valuable resource for the design of future analyses of cyanobacterial communities along coasts where oil spills may lead to environmental catastrophes; this certainly includes the Arctic (Vézina and Vincent 1997; Chap. 13).

There is another interesting perspective to consider. If oil and gas deposits are found to be abundant in the Arctic, and if it can be confirmed that these derive from carbon sequestered by the "eo" *Azolla-Anabaena azollae* symbiosis, then further observations, especially decisive ones, bear mention. The Azolla Event coincides precisely with a most acute decline in CO_2 levels, which fell from 3,500 ppm in the early Eocene to 650 ppm during this event (Pearson and Palmer 2000; Royer 2008). The net consequence was the change from "greenhouse Earth" to a planet with two frozen poles. However, if one assumes that cells of *A. azollae* represent a relatively small proportion of the total biomass of the symbiosis, albeit a critical proportion!, then any "cyanofuel" component in Arctic oil would likely to be considered minimal, as would any contribution of *A. azollae* RuBisCO to CO_2 drawdown.

Of course, were it not for the nitrogen-fixing activity of the cyanobiont the Azolla event, and the two attendant processes of carbon capture and oil biogenesis, may not have been possible, which emphasizes the critical role of cyanobacteria on carbon cycling through Earth's history. And, if conditions were supraoptimal in the Eocene Arctic Ocean for growth of *Azolla* would not planktonic cyanobacterial blooms also have been present (probably long before), in the way they are in the warmer oceans today (Fig. 16.1)?

These concepts and questions are all linked and are thus rather intractable. However, there is every suggestion to be optimistic that resilient strains or associations of cyanobacteria, grown under appropriate conditions, can be used to effect sufficient draw down of atmospheric carbon to support a biofuel industry. Although those conditions remain to be determined it is, ironically, negative oil events such as the Gulf of Mexico spill, as well as regulatory controls such as those being imposed on the aviation industry that generate the will and determination (and funding!) to pursue the much-needed research.

In this review, we raise a number of questions, concepts and perspectives regarding important relationships between cyanobacteria and oil. Of these, two questions come to the fore, and finding answers to either must involve thinking beyond the confines of extant culture collections (including the search for strains growing under continuous selective pressure of exposure to oil (Figs 16.2, 16.3 and 16.4), and the dogma for growing these organisms.

Is a global cyanofuel industry realistic?

Do cyanobacteria have the capacity to degrade hydrocarbons enzymatically?

If the answers are positive, this will close a "3.5 billion-year loop" that began with the onset of oil diagenesis and would generate further intrigue as to the origin, versatility and true potential of these fascinating organisms.

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References

Abed MM, Koster J (2005) The direct role of aerobic heterotrophic bacteria associated with cyanobacteria in the degradation of oil compounds. Int Biodeterior Biodegrad 55:29–37

Abed RMM, Safi NMD, Koster J, de Beer D, El-Nahhal Y, Rullkotter J, Garcia-Pichel F (2002) Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of petroleum compounds. Appl Environ Microbiol 68:1674–1683

Abed RM, Dobretsov S, Sudesh K (2009) Applications of cyanobacteria in biotechnology. J Appl Microbiol 106:1–12

Al-Awadhi H, Al-Hasan RH, Sorkhoh NA, Salamah S, Radwan SS (2003) Establishing oil-degrading biofilms on gravel particles and glass plates. Int Biodeterior Biodegrad 51:181–185

Al-Hasan RH, Khanafer M, Eliyas M, Radwan SS (2001) Hydrocarbon accumulation by picocyanobacteria from the Arabian Gulf. J Appl Microbiol 91:533–540

Al-Thukair AA (2002) Effect of oil pollution on euendolithic cyanobacteria of the Arabian Gulf. Environ Microbiol 4:125–129

Al-Thukair AA, Abed RMM, Mohamed L (2007) Microbial community of cyanobacteria mats in the intertidal zone of oil-polluted coast of Saudi Arabia. Mar Pollut Bull 54:173–179

Altermann W, Kazmierczak J, Oren A, Wright DT (2006) Cyanobacterial calcification and its rock-building potential during 3.5 billion years of Earth history. Geobiology 4:147–166

Angermayr SA, Hellingwerf KJ, Lindblad P, Teixeira de Mattos MJ (2009) Energy biotechnology with cyanobacteria. Curr Opin Biotechnol 20:257–263

Antic MP, Jovancicevic BS, Ilic M, Vrvic MM, Schwarzbauer J (2006) Petroleum pollutant degradation by surface water microorganisms. Environ Sci Pollut Res Int 13:320–327

Aravind L, Ponting CP (1997) The GAF domain: an evolutionary link between diverse phototransducing proteins. Trends Biochem Sci 22:458–459

Arp G, Reimer A, Reitner J (2001) Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. Science 292:1701–1704

Azar C, Lindgren K, Larson E, Mollersten K (2006) Carbon capture and storage from fossil fuels and biomass – costs and potential role in stabilizing the atmosphere. Clim Change 74:1–3

Backman J, Moran K, McInroy DB, Mayer LA, The Expedition 302 Scientists (2006) Proceedings of the integrated ocean drilling program 302. Integrated Ocean Drilling Program Management International, Inc., Edinburgh

Bailey S, Melis A, Mackey KR, Cardol P, Finazzi G, van Dijken G, Berg GM, Arrigo K, Shrager J, Grossman A (2008) Alternative photosynthetic electron flow to oxygen in marine *Synechococcus*. Biochim Biophys Acta 1777:269–276

Barth H-J (2003) The influence of cyanobacteria on oil polluted intertidal soils at the Saudi Arabian Gulf chores. Mar Pollut Bull 46:1245–1252

Beecy DJ, Kuuskraa VA (2001) Status of U.S. geologic carbon sequestration research and technology. Environ Geosci 8:152–159

Belay A (1997) Mass culture of *Spirulina* outdoors – the earthrise farms experience. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*) physiology, cell biology and biotechnology. Taylor and Francis Ltd, London, pp 79–99, 254 pp

Benemann JR (2002) A technology roadmap for greenhouse gas abatement with Microalgae. Report to the U.S. Department of Energy, National Energy Technology Laboratory, and the International Energy Agency Greenhouse Gas Abatement Programme. http://www.co2captureandstorage.info/networks/networks.htm2003. Accessed 8 Feb 2010

Benemann JR (2008) Opportunities and challenges in algae biofuels production. A position paper. http://www.fao.org/uploads/media/algae_positionpaper.pdf. Accessed 8 Feb 2010

Benemann JR, Oswald WJ (1996) Final report to US DOE NETL systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass. http://www.osti.gov/bridge/servlets/purl/493389-FXQyZ2/webviewable/493389.pdf. Accessed 8 Feb 2010

Benemann JR, Goebel RP, Weissman JC, Augenstein DC (1982) Microalgae as a source of liquid fuels. Final technical report USDOE–OER. http://www.osti.gov/bridge/product.biblio.jsp?query_id=0&page=0&osti_id=6374113. Accessed 8 Feb 2010

Bird KJ, Charpentier RR, Gautier L, Houseknecht DW, Klett TR, Pitman JK, Moore TE, Schenk CJ, Tennyson ME, Wandrey, Craig J (2008) Circum-Arctic resource appraisal; estimates of undiscovered oil and gas north of the Arctic Circle. U.S. Geological Survey fact sheet 2008–3049, Version 1.0, 23 July 2008, 4 p. Initial release online at http://pubs.usgs.gov/fs/2008/3049/

Brady DL, Apel WA, Walton MR (2004) Screening of cyanobacterial species for calcification. Biotechnol Prog 20:1345–1351

Braun RL, Burnham AK (1993) Chemical reaction model for oil and gas generation from type I and Type II kerogen. Office of Scientific and Technical Information P.O. Box 62, Oak Ridge, TN 37831, pp 30

Brinkhuis H, Schouten S, Collinson ME, Sluijs A, Sinninghe Damsté JS, Dickens GR, Huber M, Cronin TM, Onodera J, Takahashi K, Bujak JP, Stein R, van der Burgh J, Eldrett JS, Harding IC, Lotter AF, Sangiorgi F, van Konijnenburg-van Cittert H, de Leeuw JW, Matthiessen J, Backman J, Moran K, The Expedition 302 Scientists (2006) Episodic fresh surface waters in the Eocene Arctic Ocean. Nature 441:606–609

Cadoret JP, Bernard O (2008) Lipid biofuel production with microalgae: potential and challenges. J Soc Biol 202:201–211

Chaillan F, Gugger M, Saliot A, Couté A, Oudot J (2006) Role of cyanobacteria in the biodegradation of crude oil by a tropical cyanobacterial mat. Chemosphere 62:1574–1582

Chenier MR, Beaumier D, Fortin N, Roy R, Driscoll BT, Lawrence JR, Greer CW (2006) Influence of nutrient inputs, hexadecane, and temporal variations on denitrification and community composition of river biofilms. Appl Environ Microbiol 72:575–584

Clark D, Heaviside J, Habib K (2004) Reservoir properties of Arab carbonates, Al Rayyan Field, offshore Qatar. Geol Soc Lond Spec Publ 235:193–232

Cohen Y (2002) Bioremediation of oil by marine microbial mats. Int Microbiol 5:189–193

Collister J, Ehrlich R, Mango F, Johnson G (2004) Modification of the petroleum system concept: origins of alkanes and isoprenoids in crude oils. AAPG Bull 88:587–611

De Oteyza TG, Grimalt JO, Diestra E, Sole A, Esteve I (2004) Changes in the composition of polar and apolar crude oil fractions under the action of *Microcoleus* consortia. Appl Microbiol Biotechnol 66:226–232

Dismukes GC, Klimov VV, Baranov SV, Kozlov YN, DasGupta J, Tyryshkin A (2001) The origin of atmospheric oxygen on Earth: the innovation of oxygenic photosynthesis. Proc Natl Acad Sci USA 98:2170–2175

Fattom A, Shilo M (1984) Hydrophobicity as an adhesion mechanism of benthic cyanobacteria. Appl Environ Microbiol 47:135–143

Fowler MG, Stasiuk LD, Hearn M, Obermajer M (2004) Evidence for *Gloeocapsomorpha prisca* in Late Devonian source rocks from Southern Alberta, Canada. Org Geochem 35:425–441

Gallego JR, González-Rojas E, Peláez AI, Sánchez J, García-Martínez MJ, Ortiz JE, Ortiz JE, Torres T, Llamas JF (2006) Natural attenuation and bioremediation of Prestige fuel oil along the Atlantic coast of Galicia (Spain). Org Geochem 37:1869–1884

Glasby GP (2006) Abiogenic origin of hydrocarbons: an historical overview. Resour Geol 56:83–96

Gold T (1999) The deep, hot biosphere. Copernicus Books, New York, 235 pp. ISBN 0-387-98546-8

Gopalan B, Katz J (2008) Formation of long tails during breakup of oil droplets mixed with dispersants in locally isotropic turbulence. Abstract ID: BAPS.2008.DFD.MH.4. 61st annual meeting of the APS Division of Fluid Dynamics, San Antonio, Texas

Hellingwerf K, Teixeira de Mattos MJ (2009) Alternative routes to biofuels: light-driven biofuel formation from CO2 and water based on the "Photanol" approach. J Biotechnol 142:87–90

Höckelmann C, Becher PG, von Reuss SH, Jüttner F (2009) Sesquiterpenes of the geosmin-producing cyanobacterium *Calothrix* PCC 7507 and their toxicity to invertebrates. Z Naturforsch 64:49–55

Huang S, Wilhelm SW, Nianzhi J, Feng C (2010) Ubiquitous cyanobacterial podoviruses in the global oceans unveiled through viral DNA polymerase gene sequences. ISME J 4(10):1243–1251. doi: http://dx.doi.org/10.1038/ismej.2010.56

Hyne NJ (2001) Nontechnical guide to petroleum geology, exploration, drilling, and production. PennWell Corporation, Tulsa, pp 4. ISBN 087814823X

Jiayu N, Jianyi H (1999) Formation and distribution of heavy oil and tar sands in China. Mar Petrol Geol 16:85–95

Jüttner F (1991) Quantitative trace analysis of volatile organic compounds. Method Enzymol 167:609–616

Kah LC, Riding R (2007) Mesoproterozoic carbon dioxide levels inferred from calcified cyanobacteria. Geology 35:799–802

Khan NY (2008) Integrated management of pollution stress in the Gulf. In: Abuzinad AH et al (eds) Protecting the Gulf's marine ecosystem from pollution. Birkhauser Verlag AG, Basel/Boston/Berlin, 285 pp. ISBN 978-3-7643-7945-9

Kumar MS, Muralitharan G, Thajuddin N (2009) Screening of a hypersaline cyanobacterium, *Phormidium tenue*, for the degradation of aromatic hydrocarbons: naphthalene and anthracene. Biotechnol Lett 31:1863–1866

Kvenvolden KA (2006) Organic geochemistry – a retrospective of its first 70 years. Org Geochem 37:1–11

Lee BD, Apel WA, Walton MR (2004) Screening of cyanobacterial species for calcification. Biotechnol Prog 20:1345–1351

Lida T, Waki T, Nakamura K, Mukouzaka Y, Kudo T (2009) The GAF-like-domain containing transcriptional regulator DfdR is a sensor protein for dibenzofuran and several hydrophobic aromatic compounds. J Bacteriol 191:123–134

Lidz B, Gibbons H (2008) Research on whitings (floating patches of calcium carbonate mud) leads to possible explanation of immense middle east oil deposits. Sound Waves Monthly Newsletter http://soundwaves.usgs.gov/2008/07/research.html. Accessed 9 Feb 2010

Liu X, Curtis R (2009) Neutral lipid producing cyanobacteria. File Number M9-017, Arizona State University, Tempe

Llirós M, Gaju N, de Oteyza TG, Grimalt JO, Esteve I, Martínez-Alonso M (2008) Microcosm experiments of oil degradation by microbial mats. II. The changes in microbial species. Sci Total Environ 393:39–49

Mattes TE, Alexander AK, Richardson PM, Munk AC, Han CS, Stothard P, Coleman NV (2008) The genome of *Polaromonas* sp. strain JS666: insights into the evolution of a hydrocarbon- and xenobiotic-degrading bacterium, and features of relevance to biotechnology. Appl Environ Microbiol 74:6405–6416

Minas W, Gunkel W (1995) Oil pollution in the North Sea – a microbiological point of view. Helgol Mar Res 49:143–158

Mühling M, Belay A, Whitton BA (2005) Screening *Arthrospira* (Spirulina) strains for heterotrophy. J Appl Phycol 17:129–135

Murrell MC, Lores EM (2004) Phytoplankton and zooplankton seasonal dynamics in a subtropical estuary: importance of cyanobacteria. J Plankton Res 26(3):71–382

National Academy of Sciences (2009) Liquid transportation fuels from coal and biomass; technological status, costs, and environmental impacts. National Academies Press, Washington, DC, p 388

National Geographic Special Issue March (2009) Repowering the planet. National Geographic Society, Washington, DC, pp 96

Nobles Jr DR, Brown Jr RM (2008) Production and secretion of glucose in photosynthetic prokaryotes (Cyanobacteria). US Patent Application Publication US 2008/0085520 A1

Novis PM, Whitehead D, Gregorich EG, Hunt JE, Sparrow AD, Hopkins DW, Elberling B, Greenfield LG (2007) Annual carbon fixation in terrestrial populations of *Nostoc commune* (Cyanobacteria) from an Antarctic dry valley is driven by temperature regime. Glob Change Biol 13:1224–1237

NREL (2009) Algal Biofuels R&D at NREL. National Renewable Energy Laboratory, Golden, 3 pp. Available at http://www.nrel.gov/biomass/proj_microalgal_biofuels.html. Accessed 8 Feb 2010

Pacific Rim Summit on Industrial Biotechnology and Bioenergy in Honolulu (2009) http://www.highbeam.com/doc/1G1-169737441.html. Accessed 8 Feb 2010

Pearson PN, Palmer MR (2000) Atmospheric carbon dioxide concentrations over the past 60 million years. Nature 406:695–699

Pentecost A (2005) Travertine. Springer, Dordrecht, 446 pp

Potts M (1979) Ethylene production in a hot brine environment. Arch Hydrobiol 87:198–204

Potts M (1984) Nitrogen fixation in mangrove forests. In: Por FD (ed) Hydrobiology of the mangal. Dr W Junk Publishing Co, The Hague, pp 155–162

Radwan SS, Al-Hasan RH (2000) Oil pollution and cyanobacteria. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer Academic Publishers, New York, pp 307–319, 704 pp

Raghukumar C, Vipparty V, David JJ, Chandramohan D (2001) Degradation of crude oil by marine cyanobacteria. Appl Microbiol Biotechnol 57:433–436

Riding R (2006) Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic-Cambrian changes in atmospheric composition. Geobiology 4:299–316

Rocap G, Larimer FW, Lamerdin J, Malfatti S, Chain P, Ahlgren NA, Arellano A, Coleman M, Hauser L, Hess WR, Johnson ZI, Land M, Lindell D, Post AF, Regala W, Shah M, Shaw SL, Steglich C, Sullivan MB, Ting CS, Tolonen A, Webb EA, Zinser ER, Chisholm SW (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature 424: 1042–1047

Roeselers G, van Loosdrecht MCM, Muyzer G (2008) Phototrophic biofilms and their potential applications. J Appl Phycol 20:227–235

Royer DL (2008) Linkages between CO_2, climate, and evolution in deep time. Proc Natl Acad Sci USA 105:407–408

Sanchez O, Diestra E, Esteve I, Mas J (2005) Molecular characterization of an oil-degrading cyanobacterial consortium. Microb Ecol 50:580–588

Sanchez O, Ferrera I, Vigues N, de Oteya TG, Grimalt J, Mas J (2006) Role of cyanobacteria in oil biodegradation by microbial mats. Int Biodeterior Biodegrad 58:186–195

Schulze-Lam S, Harauz G, Beveridge TJ (1992) Participation of a cyanobacterial S layer in fine-grain mineral formation. J Bacteriol 24:7971–7981

Schwartzman DW, Caldiera K (2002) Cyanobacterial emergence at 2.8 GYA and greenhouse feedbacks. Abstract 105–1 of Annual meeting of the geological society of America, Denver, CO

Seddon JRT, Mullin T (2007) The motion of a prolate ellipsoid in a rotating Stokes flow. J Fluid Mech 583:123–132

Sheehan J, Dunahay T, Benemann J, Roessler P (1998) Look back at the U.S. Department of Energy's Aquatic Species Program: biodiesel from algae; close-out report. NREL Report No. TP-580-24190, 325 pp. http://www.nrel.gov/docs/legosti/fy98/24190.pdf. Accessed 8 Feb 2010

Shilo M, Ali F (1987) Cyanobacterium-produced bioemulsifier composition and solution thereof. United States Patent 4693842

Speight JG (1999) The chemistry and technology of petroleum. Marcel Dekker, New York, pp 215–216, 934 pp. ISBN 0824702174

Stein R (2006) The Paleocene-Eocene ("Greenhouse") Arctic Ocean paleoenvironment: implications from organic-carbon and biomarker records (IODP-ACEX Expedition 302). Geophys Res Abstr 8:06718. http://www.cosis.net/abstracts/EGU06/06718/EGU06-J-06718-1.pdf. Retrieved 16 Oct 2007

Stockner JG, Callieri C, Cronberg G (2000) Picoplankton and other non-bloomforming cyanobacteria in lakes. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 195–231, 669 pp

Sundquist ET, Burruss RC, Faulkner SP, Gleason RA, Harden JW, Kharaka YK, Tieszen LL, Waldrop MP (2008) Carbon sequestration to mitigate climate change: U.S. Geological Survey, fact sheet 2008–3097, 4 pp

Taketani RG, dos Santos HF, van Elsas JD, Rosado AS (2009) Characterisation of the effect of a simulated hydrocarbon spill on diazotrophs in mangrove sediment mesocosm. Appl Environ Microbiol 96:343–354

Tanaka D, Tanaka S, Yamashiro Y, Nakamura S (2008) Distribution of oil-degrading bacteria in coastal seawater, Toyama Bay, Japan. Environ Toxicol 23:563–569

The International Tanker Owners Pollution Federation Limited – ITOPF Ltd (2009) ITOPF Ltd, London. http://www.itopf.com/information-services/. Accessed 8 Feb 2010

Thomas AD, Hoon S, Linton PE (2008) Carbon dioxide fluxes from cyanobacteria crusted soils in the Kalahari. Appl Soil Ecol 39:254–263

Torres S, Fjetland CR, Lammers PJ (2005) Alkane-induced expression, substrate binding profile, and immunolocalization of a cytochrome P450 encoded on the *nifD* excision element of *Anabaena* 7120. BMC Microbiol 5:16

UNEP (2006) United Nations environment programme's division for environmental conventions. Can carbon dioxide storage help cut greenhouse emissions? Information Unit for Conventions, International Environment House, Geneva, 24 pp

Van Hamme JD, Singh A, Ward OP (2003) Recent advances in petroleum microbiology. Microbiol Mol Biol Rev 67:503–549

Vézina S, Vincent WF (1997) Arctic cyanobacteria and limnological properties of their environment: Bylot Island, Northwest Territories, Canada. (73°N, 80°W). Polar Biol 17:523–534

Zehr JP, Bench SR, Carter BJ, Hewson I, Niazi F, Shi T, Tripp HJ, Affourit JP (2008) Globally distributed uncultivated oceanic N_2-fixing cyanobacteria lack oxygenic photosystem II. Science 322:1110–1112

Part II

Physiological Ecology

Carbon

17

John A. Raven

Contents

Summary

All cyanobacteria are actually or potentially photolithotrophic, with the exception of a recently discovered non-auotrophic free-living diazotroph which is presumably a (photo-) organotroph. Photolithotrophy involves CO_2 assimilation by Form 1A or Form 1B Rubiscos with low affinity for CO_2 and a small discrimination between CO_2 and O_2 and, at present CO_2 levels, invariably involves an inorganic carbon concentrating mechanism (CCM). About half of the cyanobacterial strains tested are facultatively photo-organotrophic, a few of which are also facultative chemo-organotrophs; the rest are obligate photolithotrophs. In the natural environment the best-established cases of photo- or chemo-organotrophy are in symbioses of diazotrophic cyanobacteria with organisms that are already photosynthetic. The quantitative contribution of dissolved organic matter to otherwise photolithotrophically growing cyanobacteria is unclear. Extent cyanobacteria are involved in both biologically mediated calcification (direct role of the organism) and biologically related calcification (indirect role of the organism). The timing of the evolution of cyanobacterial CCM is unclear: the CCM probably evolved in low-CO_2 episodes in the late Neoproterozoic or the Carboniferous, with spread to all cyanobacteria in the already established major clades by horizontal gene transfer. Cyanobacteria may be the last surviving photolithotrophs as the sun emits more energy and (by whatever mechanism) there is a decreased greenhouse gas, including CO_2, content, of the atmosphere.

17.1 Introduction

Cyanobacteria are very important in the global biogeochemical carbon cycle, mainly through their autotrophic inorganic carbon assimilation coupled to oxygenic photosynthesis: essentially all free-living cyanobacteria are thus photolithotrophs, i.e. obtain energy from light and carbon from inorganic carbon (Table 17.1). They are especially important in aquatic

J.A. Raven (✉)
Division of Plant Sciences, University of Dundee at TJHI,
The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK
e-mail: j.a.raven@dundee.ac.uk

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Table 17.1 Trophic categories of cyanobacteria

Trophic category	Definition	Known distribution
Photolithotrophy	Energy from photons. Carbon from inorganic carbon	All but one known free-living cyanobacterium growing in the light. Illuminated cyanobacteria in symbiosis with non-photosynthetic hosts
Photo-organotrophy	Energy from photons. Carbon from organic carbon	A non-autotrophic free-living diazotrophic cyanobacterium. Illuminated diazotrophic cyanobacteria in symbiosis with photo-lithotrophic hosts. Minor contribution to free-living photolithotrophic cyanobacteria
Chemo-organotrophy	Energy and carbon from organic carbon	Diazotrophic cyanobacteria in light or dark in symbiosis with photolithotrophic hosts

For more detail, see text

ecosystems where they can be the dominant primary producers in some areas. Gadd and Raven (2010) suggest that not more than half of the >50 PgC per year of primary protection in the ocean, mainly in the plankton, is attributable to cyanobacteria, with almost all of the rest from oxygenic photosynthesis by eukaryotic organisms. Only a very small fraction of the primary productivity in the ocean results from chemolithotrophy plus non-oxygenic phototrophic autotrophs, 0.13% (Raven 2009) – 0.17% (Johnson et al. 2009). In inland waters cyanobacteria are very important in freshwaters, dominant in many carbonate lakes, but rare in hypersaline waters relative to eukaryotes.

The mechanism of inorganic carbon acquisition in cyanobacteria involves inorganic carbon concentrating mechanisms (CCMs), and is now known in considerable detail (Badger et al. 2006; Price et al. 2008), and will be discussed as far as it is relevant to the ecology of the organisms. There have been many recent advances in our knowledge and understanding of the spatial and temporal variations in inorganic carbon concentration and speciation in natural water bodies, and these are discussed in the context of the range of inorganic transporters and the regulation of their expression. Little is known of the interaction of the cyanobacterial CCMs with the availability of other resources, even by the standards of the limited knowledge available for eukaryotic algae (Giordano et al. 2005; Raven et al. 2005), and the range of required additional knowledge is indicated.

Cyanobacteria also occur in symbiosis with non-photosynthetic organisms (e.g. fungi, sponges: Chap. 23) where they supply all the inorganic and energy needs of the symbiosis (Table 17.1). In some cases the cyanobionts also fix N_2. Among the points considered are the contribution of these symbioses to global, and local, primary productivity.

While most of the organic matter generated in photosynthesis in cyanobacteria is retained by the cells, there is significant loss of not only respiratory CO_2 but also a range of organic molecules of a variety of sizes, from glycolate to transparent exocellular polysaccharides. Such losses are in part made up for in some cyanobacteria by the uptake of organic matter (Table 17.1). Among the topics discussed in the paper are the extent to which such uptake could permit sapro-organotrophic growth. While there are indications that one free –living cyanobacterium obtains all its organic matter from external organic matter, it is in diazotrophic symbioses with photosynthetic organisms that such growth using organic carbon is best (if still incompletely) understood, both in terms of mechanisms and of contributions to global carbon flow, as discussed in the paper.

A further aspect of the use of carbon in extant is the role of cyanobacteria in producing calcium carbonate deposits. In most cases it seems that the mineral is associated with organic materials produced by the organisms rather than through the direct intervention of the living organisms. The paper considers this calcification by cyanobacteria in both the benthic (e.g. stromatolites) and planktonic environments.

Finally, the evolution of carbon metabolism in cyanobacteria is considered. The evolution of CCMs and of calcification is considered in relation to variations in the inorganic carbon concentration and speciation in natural waters.

17.2 Acquisition and Assimilation of Inorganic Carbon in Photolithotrophy

17.2.1 Inorganic Carbon in the Cyanobacterial Environment

The habitat with the greatest global inorganic carbon assimilation by cyanobacteria is the ocean (Table 17.2). Here the total inorganic carbon concentration is about 2.2 mol m^{-3} and a pH of about 8.2, with the concentration of the three main inorganic carbon species decreasing in the order $HCO_3^- > CO_3^{2-} > CO_2$ (Zeebe and Wolf-Gladrow 2001; see Table 17.3). Photolithotrophy is limited to the upper 100–200 m (Raven et al. 2000), and this is where cyanobacteria and eukaryotic oxygenic organisms can decrease the inorganic carbon concentration relative to the concentration deeper in the ocean, where there is net chemo-organotrophy based on organic substrates generated in the surface ocean. This surface drawdown occurs because primary productivity exceeds invasion by atmospheric CO_2 plus organic carbon recycling by respiration in the surface ocean, in part because some particulate organic carbon sediments out of the euphotic zone. The drawdown of inorganic carbon in the euphotic zone

Table 17.2 Inorganic carbon availability in major habitats occupied by photolithotrophic cyanobacteria (Based on Table 17.1 of Badger et al. (2006), modified using material from Sect. 17.2.1 and Cox et al. (1965), Raven and Samuelsson (1998) and Kühl et al. (2005))

Habitat	Common genera	Environmental characteristics	Inorganic carbon supply conditions
Oceanic plankton	*Prochlorococcus* *Synechococcus* *Crocosphaera* *Trichodesmium*	High light oligotrophic to low light higher nutrients. Tropical to (*Synechococcus*) polar	Inorganic carbon about 2 mol m^{-3}, pH 8.0–8.3; lower CO_2 solubility, pK_a values for the first and second dissociations of the inorganic carbon system, than for fresh water
Coastal marine plankton	*Synechococcus* *Oscillatoria* *Phormidium* *Trichodesmium*	Oligotrophic to eutrophic, depending on terrestrial inputs. Turbidity, dissolved material can limit light	As for ocean plankton, but more variable than in open ocean as a function of terrestrial inputs, upwellings and more intensive metabolism
Estuaries	*Anabaena* *Aphanizomenon*	Very variable in salinity, light, temperature and nutrients, leading to blooms on occasion	Inorganic carbon and pH vary with terrestrial inputs
Brackish non-estuarine plankton	*Aphanizomenon* *Anabaena* *Nodularia* *Prochlorothrix*	The Baltic Sea is the main example of a large brackish non-estuarine water body connected to the ocean	Inorganic carbon availability close to that of seawater near the connection to the North Sea. Salinity less than 3 g per kg in the Gulf of Bothnia and the Gulf of Finland, with lower inorganic carbon concentrations
Freshwater Non-bloom plankton	*Cyanobium* *Snethococcus* *Cyanothece* *Aphanocapsa* *Aphanothece* *Chroococcus* *Prochlorothrix*	Waters generally poorly buffered; nutrients from oligotrophic to eutrophic	Inorganic carbon and pH very variable seasonally and on a diel basis. Usually significant inputs of inorganic carbon from terrestrial catchment. HCO_3^- dominant with rapid photosynthesis
Freshwater bloom plankton	*Anabaena* *Aphanizomenon* *Microcystis* *Oscillatoria* *Spirulina*	Gas vacuoles, mixing and stratification can help create bloom conditions, resulting in high O_2 and pH and low inorganic carbon	Factors enumerated for non-bloom phytoplankton apply, but are accentuated under high density bloom conditions
Saline inland water plankton	*Aphanothece* *Euhalothece* *Halothece*	Salinity in excess of 3 g per kg. No cyanobacteria are able to grow in saturated brine	Inorganic carbon speciation in the most saline cyanobacterial habitats is altered more than in sea water relative to freshwater. Decreasing CO_2 solubility with increasing salinity
High-alkalinity Inland water plankton	*Spirulina* *Chroococcus* *Anabaenopsis* (*Oscillatoria* on sediments)	Relatively high to high ionic strength, high carbonate alkalinity	Inorganic carbon concentration can exceed 200 mol m^{-3}. pH up to 10.5
Marine coastal mats	*Microcoleus* *Symploca* *Schizothrix*	High light attenuation coefficient, diffusion limited so anoxia at night, high O_2 and pH and low inorganic carbon in the day; temperatures can be high	Steep opposing gradients of light, temperature, nutrients, inorganic carbon, O_2 and pH mean very variable conditions for regulation of inorganic carbon concentrating mechanisms
Hypersaline mats	*Aphanothece* *Microcoleus* *Halospirulina* *Halomicronema* *Oscillatoria* *Spirulina*	As for marine coastal mats	As for marine coastal mats
Hot spring mats	*Oscillatoria* *Chlorogloeopsis* *Synechococcus*	As for marine coastal mats	As for marine coastal mats
Terrestrial mats	*Crinalium* *Microcoleus* *Chroococcus* *Nostoc*	As for aquatic mats, with the added problem of desiccation	As for aquatic mats, with varying diffusive conductance to inorganic carbon in the gas and liquid phases depending on the degree of hydration of the mat

(continued)

Table 17.2 (continued)

Habitat	Common genera	Environmental characteristics	Inorganic carbon supply conditions
Geosiphon	*Nostoc*	Intracellular in a soil surface glomeromycote fungus	Net photosynthesis by the symbiosis involves transport of external inorganic carbon through the fungus to the *Nostoc*. Also some recycling of fungal CO_2 to the cyanobacteria
Cyanolichens	*Nostoc* *Calothrix* *Scytonema* *Gloeocapsa* *Gloeothece*	Mostly terrestrial, a few aquatic with periodic exposure to air. Cyanobacteria extracellular in fungal thallus, with cyanobacteria exposed to intercellular gas spaces in many cases	Cyanobacteria exposed to intercellular gas spaces rely on gas-phase diffusion of CO_2 to the cyanobacteria; in other cases inorganic carbon must move through the aqueous phase of the fungal thallus. Some recycling of fungal respiratory CO_2 to the cyanobacteria
Marine sponges	*Synechococcus* *Oscillatoria* *Synechocystis*	Cyanobacteria intra- or extra-cellular	Inorganic carbon supply to intracellular cyanobacteria involves movement through sponge tissue. Inorganic carbon supply to all cyanobacteria is aided by mass flow through the sponge driven by light-stimulated flagella activity. Some recycling of respiratory CO_2 from the animal to the cyanobacteria
Marine ascidians	*Prochloron* *Synechocystis* *Acaryochloris*	Cyanobacteria extracellular	Inorganic carbon supply from seawater and from animal respiration
Brackish plankton	*Aphanizomenon* *Anabaena* *Nodularia* *Prochlorothrix*	Major example of a large brackish, non-estuarine water body is the Baltic Sea	Inorganic carbon availability close to that in seawater near the connection to the North Sea; salinity less than 3 g per kg in Gulf of Bothnia and Gulf of Finland, with generally lower inorganic carbon concentration

Table 17.3 Some physicochemical attributes of carbon dioxide and other inorganic carbon species relevant to photolithotrophic cyanobacteria (Based on Table 5.1 of Raven (1984a) and Table 5.2 of Falkowski and Raven (2007))

Parameter	Value in freshwater	Value in seawater (35 g salt per kg)
Concentration of dissolved CO_2 in equilibrium with 40 Pa CO_2 in the gas phase/ mol m^{-3}	25.6 (5°C)	21.5 (5°C)
	18.3 (15°C)	15.4 (15°C)
	13.6 (25°C)	11.7 (25°C)
	10.6 (35°C)	9.3 (35°C)
pK_{a1} of the inorganic carbon system = $-\log([HCO_3^-]/[CO_2 + H_2CO_3])$	6.52 (5°C)	6.05 (5°C)
	6.42 (15°C)	6.05 (15°C)
	6.35 (25°C)	6.00 (25°C)
	6.31 (35°C)	5.97 (35°C)
pK_{a2} of the inorganic system = $-\log([CO_3^{2-}]/[HCO_3^{2-}])$	10.55 (5°C)	9.34 (5°C)
	10.43 (15°C)	9.23 (15°C)
	10.33 (25°C)	9.10 (25°C)
	10.25 (35°C)	8.95 (35°C)
Diffusion coefficient of $CO_2/m^2\ s^{-1}$	0.95×10^{-9} (0°C)	
	1.94×10^{-9} (25°C) (in gas phase, 1.04×10^{-5} (25°C))	
Diffusion coefficient of $HCO_3^-/m^2\ s^{-1}$	0.53×10^{-9} (0°C)	
	1.09×10^{-9} (25°C)	
Diffusion coefficient for $CO_3^{2-}/m^2\ s^{-1}$	0.41×10^{-9} (0°C)	
	0.80×10^{-9} (25°C)	

is greatest when other nutrients are most available, e.g. in areas influenced by riverine input, or by seasonal or permanent ocean upwellings. The inorganic carbon drawdown is much less in oligotrophic areas in the ocean, where there is a low rate of input of non-carbon nutrient elements, and a correspondingly low rate of output of particulate organic carbon by sedimentation.

Lest the impression is given that there is a solely a role for biologically-driven processes (the "Biological Pump") in determining the concentration and speciation of inorganic carbon in the euphotic zone, it is important to mention the "Solubility Pump". This Solubility Pump involves the greater solubility of CO_2 in cooler than in warmer water, so there is a tendency for invasion of CO_2 from the atmosphere into cooler water, and evasion of CO_2 from cooler water into the atmosphere (Raven and Falkowski 1999). This tendency for movement of CO_2 through the atmosphere from the tropical polar ocean to the polar surface ocean is amplified by downwellings of cooler high-latitude surface waters and upwellings into warmer low-latitude waters. Chen and Borges (2009), Doney et al. (2008), and Takahashi et al. (2009) give detailed accounts of the areal distribution of CO_2 concentration in the surface ocean, and the net fluxes of CO_2 between the surface ocean and atmosphere.

Smaller contributions to global primary are made by cyanobacteria in inland waters and on land. Freshwaters (salinity not greater than 3 g/kg water) and saline waters (salinity not less than 3) occupy approximately equal areas and volumes of inland waters (see Horne and Goldman 1994; Giordano et al. 2008). Cyanobacteria make significant contributions to primary productivity in all but the most acidic (Steinberg et al. 1998) and most saline (Chap. 15) of these

inland waters which support photolithotrophy, where eukaryotic algae are predominant primary producers. Cyanobacteria are particularly important in waters of high carbonate alkalinity (some over 200 mol-equivalents m^{-3}, which qualifies as saline) and pH (some over 10.5) (Talling 1965; Talling and Talling 1965; Talling et al. 1973; Melack and Kilham 1974; Melack 1979; Jones et al. 1998; Kompantseva et al. 2009), although diatoms are also important (Hecky and Kilham 1973). While cyanobacteria are generally held to not grow at low external pH values, Steinberg et al. (1998) report filamentous cyanobacteria growing in flooded lignite mine sites at pH 2.9, although picocyanobacteria were not found below pH 4.5. HCO_3^- would have been a very small fraction of the total inorganic carbon at these acid pH values, and especially at pH 2.9: CO_2 would have comprised more than 99.9% of the total inorganic carbon

Surface water bodies receive inputs of carbonate alkalinity from weathering on land (Berner and Berner 1996), as well as organic carbon and dissolved CO_2 in groundwater from terrestrial primary productivity. Organic carbon in soil water comes below-ground parts of plants and any above-ground plant parts that become mixed into soil, while the CO_2 comes from respiration of this organic carbon in soil, ground-water and the resulting surface water bodies. These inputs impact on the inorganic status of inland waters to a relatively greater extent than on the ocean, and result in the totality of inland waters acting as a CO_2 source to the atmosphere (Cole et al. 1994; Maberly 1996; Sobek et al. 2005a, b; Duarte et al. 2008). In the case of the Dead Sea (too saline to be a significant cyanobacterial habitat) with minimal allochthonous organic carbon inputs the present fivefold CO_2 supersaturation of surface waters relative to atmospheric equilibrium is apparently due to CO_2 release from aragonite precipitation (Barkan et al. 2001).

On land, using atmospheric CO_2 as the inorganic carbon source, free-living cyanobacteria and cyanolichens in which cyanobacteria are the only photobionts are important in some areas with periodic water availability, including some arid areas with only a short period of photosynthesis possible after early in the photoperiod before dew has evaporated.

17.2.2 Mechanisms of Inorganic Carbon Acquisition and Assimilation by Cyanobacteria

The essential features of carbon acquisition and assimilation in cyanobacteria are that they have C_3 biochemistry, i.e. the initial autotrophic carboxylase is the CO_2-assimilating Rubisco embedded in the Calvin-Benson-Bassham cycle. The Rubiscos in cyanobacteria are invariably Form I, i.e. with 8 large catalytic and 8 small regulatory subunits: usually it is Form IB but a few oligotrophic ocean cyanobacteria have Form IA (Badger et al. 2002; Scott et al. 2007). Regardless of the phylogenetic subgroup to which the Rubiscos belong, they are characterized by a high CO_2-saturated specific carboxylation rates (mol CO_2 mol^{-1} Rubisco s^{-1}) and a relatively low CO_2 affinity, CO_2/O_2 selectivity and capacity to discriminate between $^{13}CO_2$ and $^{12}CO_2$ (Badger 1980; Kaplan et al. 1980; Badger et al. 2002, 2006; Giordano et al. 2005; Tcherkez et al. 2006; Price et al. 2008; Raven 2009).

The kinetic characteristics of the cyanobacterial Rubiscos are such that diffusive entry of CO_2 from an air-equilibrium solution to Rubisco would give no, or negligible, net photosynthesis (Badger 1980; Kaplan et al. 1980; Scott et al. 2007). All cyanobacteria have inorganic carbon concentrating mechanisms (CCMs) which accumulate CO_2 to higher concentrations round Rubisco in vivo than in an air-equilibrium medium. Cyanobacteria take up both CO_2 and HCO_3^- from the medium into the cytosol across the cell membrane. CO_2 enters by diffusion through the lipid bilayer component of the membrane but mainly through protein channels and is converted, with OH^-, into HCO_3^- into HCO_3^- by an energized reaction on the outer surface of the thylakoid. HCO_3^- crosses the cell membrane by active transport. The cyanobacteria with Form IB Rubisco (β-cyanobacteria) have thylakoid-expressed genes that account for high- and low-affinity CO_2-based CCMs, while those with the Form IA Rubisco (α-cyanobacteria) only have the low-affinity CO_2-based CCM (Badger et al. 2006; Price et al. 2008). Similarly, β-cyanobacteria have a high-affinity HCO_3^- transporter and one or more low-affinity HCO_3^- transporters, while α-cyanobacteria only have a low-affinity HCO_3^- transporter (Badger et al. 2006; Price et al. 2008). The result is a higher concentration of HCO_3^- in the cytosol that in the medium. The active Rubisco of cyanobacteria is all in protein-coated bodies termed carboxysomes, as is one or more forms of carbonic anhydrase. The protein coat is permeable to anions, and the anion stoichiometry (with anionic charges rounded to integers) 1 $RuBP^{2-}$ and 1 HCO_3^- enter the carboxysome. There the HCO_3^- is converted by carbonic anhydrase to CO_2, which is, with 1 H_2O and 1 $RuBP^{2-}$, into 2 PGA^-. The 2 PGA^-, with the 1 OH^-, moves to the cytosol, where the 2 PGA^- are used in the remaining reactions of the Calvin-Benson-Bassham cycle to produce 1 $RuBP^{2-}$, used in another Rubisco carboxylase reaction, and the 1C in the reduced, neutral C product denoted 1 (CH_2O). The fraction of the 1 OH^- from the carboxysome that is equivalent to the inorganic C entering the cell as CO_2 is used in another round of energized conversion of CO_2 to HCO_3^- on the thylakoid, while the fraction of the OH^- equivalent to HCO_3^- entry across the plasmalemma is lost to the medium. This sequence accounts for charge and acid-base-balance, and the overall reaction is equivalent to the conversion of one external CO_2 to one (CH_2O).

The stoichiometry of this scheme does not take into account any leakage of CO_2 from the carboxysome, and also

assumes that the accumulation of CO_2 in the carboxysomes is sufficient to cause complete competitive suppression of the oxygenase activity of Rubisco. Neither of these assumptions is correct. How CCMs minimize leakage is still incompletely understood in quantitative terms: clearly there is a significant CO_2 permeability of the cell membrane because this is part of the involvement of external CO_2 in the CCM (Badger et al. 2006; Price et al. 2008). As for oxygenase activity of Rubisco, it is now clear that there is invariably residual oxygenase activity that is not suppressed by the CCM, and that there is an apparently unique means of metabolizing phosphoglycolate to sugar phosphates that is a mixture of the plant photorespiratory carbon oxidation cycle and the bacterial glycerate (tartronic semialdehyde) pathway, and they can also completely convert glycolate to CO_2 via glyoxylate, oxalate and formate (Eisenhut et al. 2006, 2008). A triple mutant lacking all three pathways of glycolate metabolism was lethal for growth at air levels of CO_2 (Eisenhut et al. 2008).

A final point about the cell physiology of cyanobacterial photosynthetic inorganic carbon assimilation is that the both Rubiscos (cyanobacterial Form 1A and cyanobacterial Form 1B) have relatively low discrimination between $^{13}CO_2$ and $^{12}CO_2$ compared to the Form 1B Rubisco in embryophytic plants and the Form 1D Rubisco in eukaryotic algae with their higher CO_2/O_2 selectivity, higher CO_2 affinities and lower CO_2–saturated specific reaction than the cyanobacterial enzymes (Tcherkez et al. 2006; Scott et al. 2007). Taking this into account, the carbon isotope ratio of organic matter in free-living cyanobacteria and the symbioses in which cyanobacteria supply all or almost all the organic carbon can, with other evidence, help to indicate the extent of leakage from the CCM and the extent of limitation by diffusion of inorganic carbon (Raven et al. 2002).

17.2.3 Regulation of the CCM

It is now clear that CCM expression in β-cyanobacteria is a function of the inorganic carbon concentration inside the cells, with a minor role for O_2 concentration, rather than the external concentration of CO_2 which regulates the CCM in the diatoms and green algae that have been tested (Badger and Price 2003; Giordano et al. 2005; Woodger et al. 2005; Badger et al. 2006; Hammer et al. 2006; Raven 2006; Price et al. 2008; Lieman-Hurwtiz et al. 2009). The α-cyanobacteria only have a low affinity CO_2-based CCM, and a low-affinity HCO_3^- based CCM: these are constitutively expressed (Badger and Price 2003; Badger et al. 2006; Price et al. 2008). The occurrence of acclimation of CCMs and of purely constitutive CCMs in, respectively, β- and α-cyanobacteria agrees in broad terms with the habitats in which the organisms are found (Table 17.1 of Badger et al. 2006).

In eukaryotic algae CCMs are regulated not just by the inorganic carbon availability but also by the availability of such other resources as PAR, N, P and Fe (Raven 1984a, b; Giordano et al. 2005; Raven et al. 2005; Xu and Gao 2009; Hu and Zhou 2010), as well as UVB (Giordano et al. 2005; Raven et al. 2008a; Xu and Gao 2009). For cyanobacteria the only other environmental factors that have been tested for effects on CCM expression are PAR and UVB and, among nutrient elements, Fe.

Beardall (1991) showed for *Anabaena* that low PAR irradiance decreases CCM expression relative to the light-saturated conditions usually used for work on CCMs, in a manner similar to that found in eukaryotic algae (Giordano et al. 2005). Raven et al. (2000) suggest that decreased expression of CCMs at low irradiances for growth could be related to the probably increase in energy cost for active transport per net C assimilated at low irradiances where leakage of accumulated inorganic C becomes a larger fraction of the gross inorganic C efflux. This contrasts with the constant ratio of oxygenase to carboxylase activity of Rubisco for given internal (intracarboxysome for cyanobacteria) CO_2 and O_2; while a decreased expression of the CCM at low irradiances would mean a lower internal CO_2:O_2 ratio and hence a higher energy cost for Rubisco oxygenase and glycolate metabolism, there could still be an energy saving relative to leakage from a more highly expressed CCM (Raven et al. 2000). Poza-Carrión et al. (2001) examined the effects of fluctuating irradiance, pH and inorganic C on photosynthesis in *Nostoc* sp., though not in the context of CCM functioning. Campbell and colleagues (MacKenzie et al. 2004, 2005a, b; Burns et al. 2005; MacKenzie and Campbell 2005; Burns et al. 2006) examined the influence of the acclimation state of the CCM and inorganic C availability in *Synechococcus elongates* on the stoichiometry and performance of the thylakoid reactions of photosynthesis. Later work (Brown et al. 2008) on *Trichodesmium* photosynthetic flux capacity and acclimation costs have not been extended from thylakoid reactions and Rubisco to the CCM. Again, the CCM is generally taken as a given here, and the focus is on the effects of the inorganic carbon condition on the thylakoid reactions.

Song and Qiu (2007) examined the effect of UVB on the CCM of *Microcystis aeruginosa*: while UVB altered the contribution of the different components of the CCM, there was very little change in the overall inorganic C affinity for CCM, although there were fewer carboxysome per cell. For eukaryotic algae there are several studies, with results for different species ranging from a lower sensitivity of the CCM than of the downstream reactions of photosynthesis to the reverse (Giordano et al. 2005; Raven et al. 2008a).

Fu et al. (2008) examined the effect of elevated CO_2 on the growth of Fe-replete and Fe-deficient cultures of the unicellular marine diazotroph *Crocosphaera*, and found that

added CO_2 increased the growth rate of Fe-replete but not of Fe-deficient cultures. Further work is needed to examine effects on components of the CCM.

17.2.4 CO_2 and pH Dependence of Photosynthesis and Growth

At least the β-cyanobacteria have the capacity to express CCMs that allow photosynthesis and growth to be saturated by CO_2 at well below the present (temperature-dependent) air-equilibrium concentrations, and even α-cyanobacteria are CO_2 –saturated for growth (cell division) at present air-equilibrium concentrations (Kaplan et al. 1980; Kaplan and Reinhold 1999; Palinska et al. 2002; Badger and Price 2003, 2006; Price et al. 2008; Fu et al. 2007). This does not prevent increased CO_2 from increasing photosynthetic rate and carbon per cell in marine *Prochlorococcus* and *Synechococcus* (Fu et al. 2007), and may also increase the excretion of dissolved organic carbon (Raven et al. 2005). The organisms used in these studies are filaments or unicells grown in suspension, and so have minimal boundary layers (~1–10 μm), especially if the medium in which the inorganic carbon affinity is measured is stirred. However, not all such organisms are in organic-carbon saturated for growth in their natural environment: an example is the unicellular marine diazotroph *Crocosphaera* , at least when growth is not Fe-limited (Fu et al. 2008).

Since larger organisms in water have thicker diffusion boundary layers, we would expect that cyanobacteria that form colonies (Beardall et al. 2009), or form photosynthetic symbioses with larger, non- photosynthetic organisms (Raven 1993, 1999; Usher et al. 2007) would have lower affinities for inorganic carbon expressed in terms of the inorganic carbon concentration in the bulk medium. An example that has been subject to intensive recent investigation is another marine diazotroph that is not inorganic carbon-saturated for growth in air-equilibrium seawater, the filamentous, often colonial (Beardall et al. 2009) planktonic *Trichodesmium* (Hutchins et al. 2007; Levitan et al. 2007; Ramos et al. 2007; Kranz et al. 2009, 2010; Levitan et al. 2010). It is not always possible to decide if the work was done on isolated filaments or on colonies of many filaments, but the outcomes of all of the growth experiments are similar. Work on freshwater planktonic non-diazotrophic cyanobacteria has involved *Microcystis aeruginosa* (Xu and Song 2007) and *Gloeotrichia echinulata* (Vuorio et al. 2009). For three strains of *Microcystis aeruginosa* the half-saturation concentration of inorganic carbon is less than 50 mmol m^{-3} and the maximum rate increases from an external pH of 7.0–9.0 (Xu and Song 2007), so the boundary layer effect does not make the colonies inorganic carbon limited in air-equilibrium solution. The study of Vuorio et al. (2009) used the difference in stable isotope ratio in organic matter in the *Gloeotrichia ecinulata* colonies relative to that of the inorganic carbon in the lake water in which the colonies grew. For both lakes examined the largest fractionation relative to source carbon was in the smaller colonies, consistent with a greater diffusive limitation resulting from of thicker boundary layers round larger colonies. Vuorio et al. (2009) did not report the inorganic carbon affinity of growth or photosynthesis.

In benthic habitats in the sea, inland waters and on land there are cyanobacterial films and microbialites. Significant attention has been paid to colonies of the filamentous, heterocystous cyanobacterium *Nostoc* in freshwaters and on land (Dodds et al. 1995; Gao and Yu 2000; Qiu and Gao 2001, 2002a, b; Gao and Zou 2001; Gao and Ai 2004; Li and Gao 2004; Sand-Jensen 2009; Sand-Jensen et al. 2009). We deal first with the aquatic examples, since this is the ancestral condition. Li and Gao (2004) examined the benthic colonies of *Nostoc sphaeroides* found in paddy fields, and found that the inorganic carbon affinity decreased with increasing colony size.

Raun et al. (2009), Sand-Jensen (2009), and Sand-Jensen et al. (2009) examined inorganic carbon acquisition by approximately spherical 10–50 mm diameter colonies of the aquatic benthic *Nostoc zetterstedtii* from soft-water lakes in Europe. The paper does not directly report the dependence of photosynthesis or growth on external inorganic carbon: there is a large accumulation of inorganic carbon within the colonies that complicates this relationship. Even on a whole colony basis the accumulation of inorganic carbon over the external concentration (≤1 mmol m^{-3}) is up to 150-fold: in the absence of estimates of the fraction of colony volume occupied by cells the intracellular concentration of inorganic carbon cannot be determined but since the filaments are largely confined to the peripheral 2 mm of the colony the intracellular concentration must be at least 1,500-fold the external value, i.e. to ≥1.5 mol $m^{-3,}$ if all the accumulated inorganic carbon is in the cells. Sand-Jensen et al. (2009) comment that accumulation ratio of inorganic carbon for unicellular or filamentous non-colonial cyanobacteria is 500–1,000-fold (Kaplan et al. 1980; Badger and Price 2003). The extracellular oxygen concentration within photosynthesizing colonies (Sand-Jensen et al. 2009) is less than for some bulky photosynthetic tissues (Raven and Larkum 2007) despite the lengthy diffusion path for oxygen efflux, presumably the result of the low photosynthetic rates. The main function of the colony in the carbon economy of the organism seems to be a major restriction on the loss of respiratory inorganic carbon in the dark, analogous to the Crassulacean Acid Metabolism in some vascular plants: the restricted access to the cells of external inorganic carbon (and other nutrients) is offset by recirculation of endogenous inorganic carbon. The observed, high natural abundance ratio of ^{13}C to ^{12}C of organic matter in the *Nostoc* relative to source

inorganic carbon is consistent with very little leakage from the accumulated inorganic carbon pool (Sand-Jensen et al. 2009). The colonies also have a very large package effect for absorption of photosynthetically active radiation which is essentially independent of colony size (Sand-Jensen et al. 2009; cf. Li and Gao 2004) meaning a small relative return per unit time in a given PAR field on the investment in photosynthetic machinery relative to smaller organisms (see Raven 1984a, b). Furthermore, there is a higher dark respiration per unit photosynthesis than in smaller cyanobacteria and a large allocation of photosynthate to extracellular matrix (Sand-Jensen et al. 2009). The photosynthetic and respiratory rates show that it must take several years for a colony to grow to 50 mm diameter; the correspondingly low mortality rate is consistent with the absence of invertebrate or vertebrate grazers (Sand-Jensen et al. 2009). Clearly the *Nostoc* colonies can compete with eukaryotic macroalgae as well as mosses and vascular plants.

The carbon dioxide dependence of photosynthesis and growth in terrestrial *Nostoc flagelliforme* mats have been investigated by Gao and Yu (2000) and Qiu and Gao (2001, 2002a): both processes are CO_2-limited at present atmospheric levels. The highest rates occur at intermediate water contents: at high water contents the rate is limited by diffusion of CO_2 through the surface water layer, while at lower water contents the rate is limited by desiccation effects on metabolism.

Microbial mats in saline waters dominated by cyanobacteria (*Calothrix crustacea* or *Lyngbya aestuarii*) or containing diatoms as well as a cyanobacterium (*Microcoleus chthonoplastes*) were studied by Rothschild and Mancinelli (1990) as models for stromatolites, using the ^{14}C-inorganic C technique. 2 mol m^{-3} dissolved inorganic carbon gives photosynthetic rates of 0.1–0.2 of the inorganic C-saturated rate for submerged mats, while present-day atmospheric CO_2 concentrations give rates about 0.01 of the CO_2-saturated rate for emersed mats; the CO_2-saturated rates are somewhat lower when measured on emersed mats than when measured on submersed mats, an effect more pronounced at low substrate concentrations. However, the rates here, especially at rate-limiting inorganic carbon concentrations, are under-estimates by 2–5-fold when based (as in Rothschild and Mancinelli 1990) on the bulk phase inorganic ^{14}C specific activity rather than that in the interstitial water in the mat (Revsbeck et al. 1981). Nevertheless, it is likely that cyanobacterial mats and stromatolites are not saturated with inorganic carbon is media with 2 mol m^{-3} inorganic C in equilibrium with the present atmosphere (Raven et al. 2008a).

The intertidal cyanolichen *Lichina pygmaea* is inorganic carbon saturated in both the present atmosphere and in stirred air-equilibrium seawater (Raven et al. 1990). The *Calothrix* cyanobiont is internal to the fungal hyphae and it is possible that the fungal component has a function other than allowing inorganic C diffusion; whatever the mechanism it results in a low discrimination among carbon isotopes (Raven et al. 1990). For terrestrial cyanolichens there are typically intercellular gas spaces on which the cyanobionts abut; these gas spaces are maintained inter alia by hydrophobins (hydrophobic proteins) in the cell walls, operating in a manner analogous to that of the internal cuticle in vascular plant (and many bryophyte) sporophytes (Honegger 1998; Dyer 2002; Raven 2002; see also Raven 1986, 1993, 2003). In these cases the affinity of the cyanolichen for CO_2 is similar to that of the isolated cyanobiont, with a relatively similar extent of carbon isotope discrimination, so that both in hospice and ex hospice a CCM is involved and there is not a major additional diffusion limitation in hospice (Cowan et al. 1992; Palmqvist 1993, 2000; Palmqvist et al. 1994; Maguas et al. 1995; Smith and Griffiths 1998). The terrestrial free-living cyanobacteria lack the hydrophobic surfaces that could permit gas-phase diffusion into mats in the manner found in lichens with gas spaces, thus accounting for the gas exchange and growth characteristics of *Nostoc flagelliforme* found by Gao and Yu (2000) and Qiu and Gao (2001, 2002a) that are indicative of significant diffusive limitation. Free-living cyanobacteria thus resemble cyanolichens lacking intercellular air spaces ion terms of inorganic carbon supply, and are analagous to hornworts, which are bryophytes with no gas spaces in their gametophytes but with, in many species, a CCM (Meyer et al. 2008).

A final example of host interactions with cyanobiont inorganic carbon acquisition is that of marine tropical and warm temperate shallow-water sponges, where among the many archaeal, bacterial and eukarytic microbial associates are three clades of mutualistic photosynthetic cyanobacteria (Taylor et al. 2007; Usher et al. 2007; Lemloh et al. 2009). Raven (1993, 1999, 2003) cites evidence that, unlike non-symbiotic sponges which have similar rates of flagella-induced water flow through the organism in light and dark, sponges with photosynthetic cyanobionts have several-fold greater rates of water flow through the symbiosis in the photophase than the scotophase. This is consistent with the water flow supplying respiratory oxygen and particulate organic matter, and removing carbon dioxide and other excretory products, and egesta, in non-symbiotic sponges, but predominantly supplying inorganic carbon and other nutrient elements in the sponges with sufficient cyanobionts to provide most of the of the organic carbon needed by the symbiosis. Provision of inorganic carbon throughout the sponge is needed in view of the role of silica in dispersing photosynthetically active radiation within the organism (Brummer et al. 2008). The small discrimation between carbon isotopes in cyanosponges is consistent with some diffusion limitation of inorganic C supply and little leakage of inorganic C from pool accumulated in the cyanobiont by the CCM (Raven et al. 2002). However, more data are needed, for example of

the kind provided by Sand-Jensen and Pedersen (1994) for the freshwater *Spongilla lacustris* with *Chlorella* (Trebouxiophyceae: Chlorophyta) symbionts, where saturation of photosynthesis requires a higher concentration of inorganic carbon than occurs in air-equilibrium lake water. Sand-Jensen and Pedersen (1994) commented that none of the freshwater green algal-phagotroph symbioses examined can use HCO_3^-, and that *Spongilla lacustustris* grow faster in rivers than in lakes, suggesting that external water flow is important in supplying metabolic substrates for photosynthesis and phagotrophy and removing waste products.

The measurements of almost instantaneous effects of varied inorganic carbon supply on photosynthetic rate do not allow acclimation. Growth with different inorganic carbon supplies does show acclimation. However, no experiments have been made using long-term growth (of the order of 1,000 generations) of a cyanobacterium at varying inorganic carbon concentrations of the kind performed with *Chlamydomonas reinhardtii* to investigate the possibility of genetic adaptation (Collins and Bell 2004).

Many cyanobacteria are able to grow photolithotrophically at high pH e.g. in carbonate lakes. The maximum pH at which net photosynthesis can occur is defined by the pH compensation value (the highest pH that can be achieved in a pH drift experiment) (Allen and Spence 1981; Maberly 1983; Maberly and Spence 1983), which ideally corresponds to the compensation CO_2 concentration (the steady-state CO_2 concentration achieved by photosynthesis in a limited volume of medium containing inorganic carbon) (Birmingham and Colman 1979). The pH compensation value has been criticized as an indicator of the mechanism of inorganic carbon assimilation, e.g. because the upper limit of attainable pH could be set by pH per se rather than inorganic carbon concentration and speciation (Hansen et al. 2007); however, it can still give very useful information (Maberly et al. 2009).

The CO_2 compensation concentration in aqueous medium for the free-living freshwater cyanobacteria examined is low, as expected for organism expressing a CCM (Birmingham and Colman 1979), as well as for the intertidal cyanobacterial lichen *Lichina* (Raven et al. 1990) and a number of terrestrial cyanolichens measured in air (Maguas et al. 1995). The pH compensation value is 9.74 for *Lichina* in seawater at 5°C (Raven et al. 1990), and higher for freshwater cyanobacteria, i.e. 10.44–11.67 for 19 strains of the planktonic colonial *Microcystis aeruginosa* at 24°C (Bañares-España et al. 2006) and 10.97–11.07 for the benthic colonial *Nostoc zetterstedtii* at 15°C (Sand-Jensen et al. 2009). For the terrestrial *Nostoc flagelliforme* submerged at 20°C in water with 3.3 mol inorganic C m^{-3} to mimic rainfall on the alkaline soil on which it grows the pH compensation value was 10.8 (Gao and Zou 2001). The high values for pH compensation in freshwater media than in seawater is presumably a function of the lower pK_{a1} and pK_{a2} of the inorganic carbon system in seawater, so that the equilibrium CO_2 concentration is lower in seawater than in freshwater for a given pH in the range mentioned above for a given initial inorganic carbon concentration and alkalinity (Table 17.3; Falkowski and Raven 2007).

An extreme case of freshwater cyanobacterial exposure to CO_2 in the laboratory was reported by Thomas et al. (2005), who showed that growth could continue in 100 kPa (about one atmosphere) CO_2. The equilibrium concentration in solution at 25°C is 34 CO_2 mol m^{-3} (see Table 17.3). By contrast, the CO_2 concentration in a soda lake with 200 mol equiv m^{-3} carbonate alkalinity and pH 10.5 is only 0.8 mmol m^{-3} at 25°C, assuming sea-water salinity (Sect. 17.2.1; Table 17.3).

17.3 Acquisition and Assimilation of Organic Carbon for (Photo)Organotrophy

17.3.1 Organic Carbon in the Cyanobacterial Environment

Aquatic environments have a wide range of dissolved organic carbon compounds from terrestrial inputs, loss of organic carbon from growing primary producers, autocatalytic (including apoptotic) cell death, the influence of viral and other pathogens on organisms, and decomposition of dead particulate organic matter; many of these compounds are intractable to both biological and physicochemical processes and hence are very long-lived (Hellebust 1974; Arnon and Benner 1994; Berner and Berner 1996; Fuhrman 1999; Hansell et al. 2004; Berman-Frank et al. 2007). The extent to which the saprophytic potential of those cyanobacteria which have transporters and assimilatory enzymes for a range of simple organic compounds are expressed is not clear, with even less information on more complex organic molecules (17.3.2). Diazotrophic cyanobacteria symbiotic in photosynthetic organisms have a limited photosynthetic capacity and use one or more organic compounds from the photosynthetic host (17.3.3).

17.3.2 Organic Carbon Acquisition and Assimilation in Free-Living Cyanobacteria

The two forms of organotrophy are photo-organotrophy (energy from light; carbon from organic carbon) and chemo-organotrophy (both energy and carbon from organic carbon): see Table 17.1. The only known case in which there has to be dissolved organic carbon assimilation by a free-living cyanobacterium is the recently discovered globally distributed but as yet uncultivated marine diazotrophic organism that lacks photosystem II, and hence oxygenic photosynthesis, and autotrophic carbon metabolism (Zehr et al. 2008).

Since this organism has photosystem I, and hence can presumably generate a proton gradient and generate ATP by cyclic photophosphorylation, it can function as do the Archaea and Bacteria with bacterio-/halo-/proteo-rhodopsin and the anoxygenic aerobic bacteria containing a photosystem I-like bacteriochlorophyll-based photosynthetic apparatus, and also lack autotrophic inorganic carbon assimilation (Raven 2009). All of these organisms can in principle produce more biomass from a given quantity of a given dissolved organic substrate than can organisms lacking energy-conserving photochemistry. In the light photochemistry can generate ion gradients used in solute transport and flagellar motility, and ATP usable in a wide range of endergonic biochemical and biophysical processes, all of which would otherwise involve respiratory energy transduction using organic substrates (Raven 2009). In the diazotrophic cyanobacterium lacking photosystem II, the photochemistry could also (as in heterocysts) generate a stronger reductant for use in diazotrophy than is produced in respiratory metabolism.

For other free living cyanobacteria, with the capacity for photolithotrophy, a distinction is drawn between those that are obligate photolithotrophs and those that can grow chemo-organotrophically (Droop 1974; Zhang et al. 1998; Zubkov 2009). Obligate photolithotrophy is a subset of obligate autotrophy, including obligate chemolithotrophy and, according to some authors, methanotrophy (Kelly 1971; Rittenberg 1972; Wood et al. 2004; Zhang et al. 1998). It is clear that obligate autotrophy does not necessarily mean that organic compounds cannot be taken up and incorporated during growth in the light on inorganic compounds, although there may not be an increase in growth rate as a result of the assimilation of organic compounds, even when photosynthetic energy supply limits the growth rate. For compounds with only C, H and O this is illustrated by the uptake of glucose by *Prochlorococcus* (Gomez-Baena et al. 2008) and fructose uptake by *Anabaena* (Ungerer et al. 2008). There is also the incidental entry of organic carbon in the capacity to use organic nitrogen as N-source (e.g. Mary et al. 2008; Zubkov 2009; see Martiny et al. 2009). An exception to organic carbon entry in the use of organic nitrogen is the use of an extracellular amino-acid oxidase with uptake of the resulting ammonium, but not the N-free (for an amino-acid with only one N per molecule) 2-oxo-acid, a mechanism found in several species of marine and freshwater cyanobacteria, including *Synechococus* (Bockholt et al. 1996; Wawrick et al. 2009), *Trichodesmium* (Mulholland et al. 1998) and *Synechocystis* (Schreik et al. 2007). For the use of external organic P compounds, much evidence supports the enzymic hydrolysis of phosphate esters outside the cells with subsequent uptake of the inorganic phosphate but not necessarily of the organic moiety (Whitton et al. 2005). However, Whitton et al. (2005) also suggest that uptake of the intact phosphate esters by cyanobacteria is also possible. Phosphonates, with C-P bonds, can be used by some cyanobacteria; they are taken up intact, so organic C also enters the cells (Dyhrman and Haley 2006; Dyhrman et al. 2006; Illykchyan et al. 2009). The restricted availability of phosphonates, the restricted distribution of phosphonate use among cyanobacteria, and the low C:P ratio in phosphonates relative to the Redfield Ratio atomic C:P of 106:1, mean that the global supply of organic C to cyanobacteria from phosphonates is limited, while the occurrence of phosphate ester uptake by cyanobacteria needs further investigation.

Zhang et al. (1998) point out that only about half of the cyanobacterial strains tested are capable of photoorganotrophic or, more rarely, chemoorganotrophic growth, almost invariable with a sugar (strain-specific) as the only acceptable substrate. Chemoorganotrophy is found in, for example, strains of *Nostoc, Plectonema* and *Synechocystis* (of which strain PCC6714 was the first cyanobacterium for which the complete genome sequence was available). Zhang et al. (1998) found a correlation between the occurrence of photo- or organotrophic growth on glucose in strains of these three genera and the occurrence of active influx of glucose and the presence of the *glcP* gene coding for a proton-glucose symporter. Transfer of the *glcP* into an obligately photolithotrophic strain of *Synechocystis* did not result in the capacity for photo- or chemo-oranotrophic growth, at least over more than a short period, even when added as a replicative plasmid (Zhang et al. 1998).

For strains of the heterocystous diaotroph *Anabaena*, Ungerer et al. (2008) examined two *Anabaena* strains, one with the capacity to grow photo- or chemo-organotrophically on fructose as well as photolithotrophically, the other not. Insertion of an ABC-type (i.e. directly ATP-using) fructose active transporter gene on a replicative plasmid into the strain incapable of photo- or chemo-organotrophic growth permits chemo-organotrophic growth, but only in the present of a regulatory gene, and does not permit photo-organotrophic growth of this strain. It is clear that the causes of the various trophic modes of cyanobacteria with respect to carbon and energy sources are not simple. It is also difficult to evaluate quantitatively the ecological and evolutionary significance of the capacity for photo- or chemo-organotrophic growth on a very restricted range of organic compounds in many natural environments, an exception being cyanobacterial diazotrophs, i.e. species of *Nostoc* and, it was thought, *Anabaena*, growing in symbiosis with photosynthetic hosts (Usher et al. 2007). Ungerer et al. (2008) suggest that free-living strains of *Anabaena* that can use fructose for photo- or chemo-organotrophic growth could be derived from symbionts; however, as Ungerer et al. (2008) point out, although it was thought that the diazotrophic symbionts of *Azolla* was *Anabaena azollae*, it is now believed that the true symbiont has not yet been cultured, and there are no known symbiotic

strains of *Anabaena.* Later work by Ran et al. (2010) shows that the *Azolla* symbiont (‘*Nostoc azollae*’) has significantly fewer genes in a smaller genome than in relatives capable of independent existence, and so is not capable of independent growth.

17.3.3 Organic Carbon Acquisition and Assimilation in Diazotrophic Cyanobacterial Symbioses with Photosynthetic Hosts

Diazotrophic cyanobacterial symbioses with photosynthetic hosts occur under the soil in cycads and *Gunnera*, so they clearly cannot photosynthesise (Tredici et al. 1988; Rai et al. 2000; Black et al. 2002; Black and Osborne 2004): Table 17.1. Even when they are illuminated to varying extents (cyanobacteria in cephalodia in lichens with photosynthate from green algal photobionts, marine diatoms with *Richelia*, freshwater diatoms with small nitrogen-fixing bodies derived from cyanobacteria, liverworts, hornworts and *Azolla* with *Nostoc*) they have a limited, or no capacity for photosynthesis (Steinberg and Meeks 1989; Rai et al. 2000, 2002; Kneip et al. 2007, 2008; Adams and Duggan 2008; Wouters et al. 2009; Ran et al. 2010): Table 17.1. In the cases examined, organic carbon from the photosynthetic host is supplied to the cyanobiont as sugars such as, in embryophytes, glucose, fructose or sucrose (Rai et al. 2000, 2002). There may be similarities to the form in which organic carbon is transferred from vegetative cells to heterocysts within a heterocystous cyanobacterium (Cumino et al. 2007).

17.4 Conversion of Dissolved Inorganic Carbon into Calcium Carbonate Minerals

The cyanobacteria lack of an endomembrane system of the kind found in eukaryotes, permitting both endocytosis and exocytosis of solutions and particles (Gadd and Raven 2010; Raven and Knoll 2010). This lack means that cyanobacteria are unable to exocytose any $CaCO_3$ produced in intracellular vesicles: intracellular calcification with subsequent externalization occurs in certain eukaryotes, e.g. coccolithophores and many foraminiferans (Gadd and Raven 2010; Raven and Knoll 2010). Such $CaCO_3$ deposition as cyanobacteria are involved in is precipitated outside the cells as biominerals and organominerals, with no evidence suggesting intracellular calcification (Raven and Giordano 2009; see Lee et al. 2004; Riding 2006, 2008). The distinction between biominerals and organominerals (Perry et al. 2007) is that biominerals are produced directly by organisms by precipitation using soluble substrates in biologically mediated calcification, while organominerals are affected by organic compounds in biologically related calcification, e.g. mineral particles trapped by stromatolites. “Affected by organic compounds” opens the way to “biomimetic” structures, e.g. that stromatolite structures may occur some distance from the organism producing the organic polymer occurs (Grotzinger and Rothman 1996; Grotzinger and Knoll 1999; Mcloughlin et al. 2008). This topic is revisited when considering the evolution of how cyanobacteria interact with inorganic carbon.

The present surface ocean is almost all supersaturated with respect to all of the major crystalline forms of $CaCO_3$, i.e. aragonite, calcite and high-magnesium calcite, although this supersaturation will decrease, and become undersaturation, with increasing anthropogenic CO_2 production (Doney et al. 2009). Some inland waters are also supersaturated with $CaCO_3$ (Arp et al. 1999; Dittrich and Obst 2004; Dittrich et al. 2004). Overall, photosynthesis and net primary productivity in aquatic habitats removes CO_2 from the water, regardless of the inorganic C species entering when intracellular acid–base balance has been the taken into account. This alters the inorganic C speciation around the cells, such that CO_2 and HCO_3^- decrease, as does H^+, while CO_3^{2-} and OH^- increase, so that the saturation status of the mineral phases of $CaCO_3$ is also increased. However, this is still insufficient to cause production of the mineral phase: this needs both nucleation sites and the absence or near-absence of inhibitors, e.g. phosphates, of crystal growth: Arp et al. (1999), Dittrich and Obst (2004), Dittrich et al. (2004). Kosamu and Obst (2009), and Obst et al. (2009) suggest that the surface of picocyanobacteria can act as a template that causes nucleation, accounting for “whiting” ($CaCO_3$ particles in the water body: Riding 2006; see Chap. 16), although in other case (alkaline salt lakes) extracellular polymeric substances, mainly produced by the cyanobacteria but not necessarily still associated with them, are involved (Arp et al. 1999). A similar sequence of events presumably occurs in the production of stromatolites in shallow subtropical seawater (Shark Bay, Western Australia: Konishi et al. 2001), thrombolites in a lake with seasonally variable salinity (Lake Clifton, Western Australia) and very large (40 m tall) microbialites in Lake Van (the largest soda lake in the world) in Anatolia, Turkey (Kempe et al. 1991; Benzerana et al. 2006). However, it must be borne in mind that at least present-day calcitic microbialites in inland waters relate to non-cyanobacterial algae.

17.5 Cyanobacterial Interactions with Inorganic Carbon in the Past

Cyanobacteria have existed for at least 2.4 billion years (Rasmussen et al. 2008; cf. Brocks and Pearson 2005; Mulkadjinian et al. 2006; Tomitani et al. 2006; Falkowski

and Raven 2007; Raven et al. 2008a, b; Shi and Falkowski 2008: see Chap. 2). Over that time there have been low CO_2 episodes in the Precambrian, as judged from icehouse episodes some 2.3–2.2, 0.75 and 0.6 billion years ago as well as in the Phanerozoic (see Giordano et al. 2005; Kopp et al. 2005), as well, from non-icehouse evidence, as an episode with rather less low CO_2 some 1.3–1.4 billion years ago. In the Carboniferous about 300 million years ago there is evidence not just of glaciations and, though a number of proxies, low carbon dioxide as well. In the late Neogene, including the Pleistocene glaciations, there is evidence of cold and glacial episodes and, in parallel, proxies for low CO_2 and, for the last 800,000 years, direct evidence of atmospheric CO_2 from ice cores. Raven (1997) and Badger et al. (2002) suggested that the cyanobacterial CCM evolved in the Carboniferous. By this time the major clades of cyanobacteria were established (Tomitani et al. 2006; Shi and Falkowski 2008), so the distribution of CCMs must have involved horizontal gene transfer (HGT). This also accounts for the occurrence of Form IA Rubisco in the α-cyanobacteria (Scott et al. 2007), though the occurrence of α-carboxysomes evolved in the α-cyanobacteria is presumably the result of evolution within the clade, with occurrence of α-carboxysomes in anoxygenic phototrophs apparently a result of HGT (Badger et al. 2002). Interestingly, CCM-specific genes are not specifically mentioned in the analysis by Shi and Falkowski (2008) of the stable core and the variable shell of genes in genome evolution of cyanobacteria.

However, there is also the possibility of evolution of CCMs in earlier low-CO_2 episodes (Giordano et al. 2005; Riding 2006; Raven et al. 2008a). Riding (2006) makes a case for the origin of CCMs in parallel with the onset of sheath calcification of cyanobacteria in time period 0.7–0.57 billion years ago. CCMs, by increasing the possibility of inorganic carbon drawdown with a decreased CO_2 compensation concentration and increased pH compensation value, can cause, or increase a pre-existing, local carbonate saturation and hence favour precipitation, provided there is a nucleation catalyst and an insufficient concentration of crystal growth inhibitor to prevent crystal growth. As with the earlier suggestion of a Carboniferous origin of CCMs, the major clades of cyanobacteria had been established by the late Neoproterozoic (Tomitani et al. 2006) so horizontal gene transfer is again required to account for the universal distribution of CCMs among photolithotrophic cells of cyanobacteria. In view of suggestions as to the occurrence of Snowball Earth (or Slushball Earth) episodes during these low-CO_2 episodes, it is helpful that there are now known fossils of micro-organisms, albeit with limited taxonomic resolution, from strata of this age confirming the continuity of a diversity of organisms through this time (Corsetti et al. 2003), a continuity that imposes restrictions on the spatial extent of a Snowball Earth.

Regardless of when the cyanobacterial CCM evolved, there is the problem of how the CCM was maintained over the lengthy (>100 million year) periods between low-CO_2 episodes (Raven et al. 2008a). Habitats such as stromatolites provide a potential low-CO_2 habitat in these high-CO_2 intervals (Raven et al. 2008a), and the invocation of HGT to explain the universal occurrence among cyanobacteria of a trait that evolved well after the origin of cyanobacteria potentially accounts for the spread of CCMs to all cyanobacteria in subsequent global low-CO_2 episodes and the present universal occurrence of CCMs in photolithotrophic cells of cyanobacteria. Raven et al. (2011) suggest that interactions of CCMs with other environmental factors which vary with water temperature could help to retain CCMs in high-CO_2 episodes. Turning from "when" to "whence", the relatively late evolution of CCMs in cyanobacteria is consistent with the re-use of proteins with functions other than in CCMs in producing the structures and catalysts used in the CCM (Raven et al. 2008a, b).

For the evolution of calcification by cyanobacteria, calcium carbonate deposits are always extracellular, and it has already been pointed out that the absence of an endomembrane system of the kind found in eukaryotes that allows externalization of particles formed in intracellular vesicles means that precipitation cannot have been originally. This limits the extent to which the organism can modify the calcification environment in a manner favouring calcification in habitats that are undersaturated with respect to any solid phase of calcium carbonate (Gadd and Raven 2010; Raven and Knoll 2010). Riding (2006) considers the origin of sheath calcification, a spatially specific kind of biologically mediated calcification in cyanobacteria in the Neoproterozoic in the context of the inorganic carbon environment and UV-B and the origin of the CCM. Stromatolites significantly predate widespread sheath calcification, though it must be borne in mind that the calcium carbonate in stromatolites (used broadly to include other microbialites) could originate from organisms other than cyanobacteria, or could even be abiogenic (Riding 2008; Raven and Giordano 2009; Gadd and Raven 2010). Phanerozoic stromatolites and their relationship to cyanobacteria are considered by Kah and Riding (2007), Riding (2008, 2009), and Oliveri et al. (2010): see also Breecker et al. (2010).

17.6 Cyanobacteria and Inorganic Carbon in the Future

Earlier in this article work on the effects of increased CO_2 to the levels predicted by the end of the present century, showing that some cyanobacteria (e.g. the marine picoplanktonic *Prochlorococcus, Synechococcus*) show no increase in growth rate (rate of cell division) in high CO_2 but an increase in

organic C per cell, while others (e.g. the marine diazotrophs *Crocosphaera* and *Trichodesmium*) show an increase in the rate of cell division with increased CO_2. As was also indicated earlier, these studies relate to the acclimation of extant genomes, not to the possibilities of genetic adaptation (cf. Collins and Bell 2004). This makes it difficult to predict what will happen to the properties, and regulation, of the cyanobacterial CCM during the 2100s, especially since there are a variety of methods for experimentally simulating the effects of increased CO_2 on aquatic photolithotrophy each with varying degrees of realism and technical difficulty (Rost et al. 2008; Gattuso and Lavigne 2009; Hurd et al. 2009; Schulz et al. 2009; Shi et al. 2009). The situation is made more difficult for cyanobacteria in their natural environment because there are several other environmental changes, e.g. increased temperature (addressed in relation to CO_2 increase in the study by Fu et al. 2007), altered availability of other nutrients (addressed in relation to Fe and its interactions with CO_2) and altered mixed layer depth and hence mean PAR: (see Finkel et al. 2010; Raven et al. 2011). There is also a relative lack of studies of the interaction of increased CO_2 with other environmental changes (discussed above), e.g. changes in nutrient supply (except Fe: Fu et al. 2007): other studies deal with effects of PAR (Beardall 1991) and UV-B (Song and Qiu 2007) on functioning of the CCM rather than effects of enhanced CO_2 for interacting with changed electromagnetic radiation. Here we know very little about acclimatory interactions between CO_2 increase and changes in other environmental factors, and nothing about genetic adaptation.

In the far distant future the increasing radiation output from the sun will eventually boil off the oceans, destroying the (photosynthetic) biosphere, then all life, well before the sun turns supernova. A decreased atmospheric content of greenhouse gases could delay the heat death of the (surface) biosphere. Lovelock and Whitfield (1982) considered the effects of such a reduction on the quantity of the major (in an oxygenated atmosphere) greenhouse gas CO_2 on the potential for photosynthesis in the context of C_3 terrestrial plants with their relatively high CO_2 compensation concentration and low in vivo affinity for CO_2. Caldeira and Kasting (1992) extend this to terrestrial C_4 plants with their lower CO_2 compensation concentration and their higher in vivo affinity for CO_2 and suggest that this mechanism could extend the life-span of the (photosynthetic) biosphere by a few 100 million years. Extending this to CCMs other than those of C_4 land plants, Raven et al. (2008b) speak of such prolongation of the life of the (photosynthetically driven) biosphere as "salvation through CCMs", noting that this is independent of how the CO_2 content of the biosphere is decreased: a Gaian feedback is not an essential part of the suggestion, although clearly some mechanism is needed. Further discussion on the end of the biosphere is to be found in Sherrat and Wilkinson (2009).

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References

Adams DG, Duggan PS (2008) Cyanobacterium-bryophyte symbioses. J Exp Bot 59:1047–1058

Allen ED, Spence DHN (1981) The differential ability of aquatic plants to utilize the inorganic carbon supply in fresh waters. New Phytol 87:269–283

Arnon RMW, Benner R (1994) Rapid cycling of high-molecular-weight organic matter in the ocean. Nature 369:549–552

Arp G, Reimer A, Reitner J (1999) Calcification in cyanobacterial biofilms of alkaline salt lakes. Eur J Phycol 34:393–403

Badger MR (1980) Kinetic properties of ribulose 1,5-bisphosphate carboxylase-oxygenase from *Anabaena variabilis*. Arch Biochem Biophys 201:247–254

Badger M-R, Price GD (2003) CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. J Exp Bot 54:609–622

Badger MR, Hanson M, Price GD (2002) Evolution and diversity of CO_2-concentrating mechanisms in cyanobacteria. Funct Plant Biol 29:161–173

Badger MR, Price GD, Long BM, Woodger FJ (2006) The environmental plasticity and ecological genomics of the cyanobacterial CO_2 concentrating mechanism. J Exp Bot 57:249–265

Bañares-España E, López-Rodas V, Salgado C, Dostas E, Flores-Moya A (2006) Inter-strain variability in the photosynthetic use of inorganic carbon, exemplified by the pH compensation point, in the cyanobacterium *Microcystis aeruginosa*. Aquat Bot 85:159–162

Barkan E, Luz B, Lazar B (2001) Dynamics of the carbon dioxide system in the Dead Sea. Geochim Cosmochim Acta 65:355–368

Beardall J (1991) Effects of photon flux-density on the CO_2-concentrating mechanism of the cyanobacterium *Anabaena variabilis*. J Plankton Res 13:S133–S141

Beardall J, Allen D, Bragg J, Finkel ZV, Flynn KV, Quigg A, Rees TAV, Richardson A, Raven JA (2009) Allometry and stoichometry of unicellular, colonial and multicellular phytoplankton. New Phytol 181:295–309

Benzerana K, Menguy N, López-Garcia P, Yoon T-H, Vazmiercak J, Tylsczak T, Guyot F, Brown GE Jr (2006) Nanoscale detection of organic signatures in carbonate microbialites. Proc Natl Acad Sci USA 103:9440–9445

Berman-Frank I, Rosenberg G, Levitan O, Hanraty L, Mari X (2007) Coupling between autocatalytic cell death and transparent exopolymeric particle production in the marine cyanobacterium *Trichodesmium*. Environ Microbiol 9:1415–1422

Berner EK, Berner RA (1996) Global environment. Water, air and geochemical cycles. Prentice Hall, Upper Saddle River

Birmingham BC, Colman B (1979) Measurement of carbon dioxide compensation points for freshwater algae. Plant Physiol 64:892–895

Black KG, Osborne BA (2004) An assessment of photosynthetic downregulation in cyanobacteria from the *Gunnera-Nostoc* symbiosis. New Phytol 162:125–132

Black KG, Parsons R, Osborne BA (2002) Uptake and metabolism of glucose in the *Nostoc-Gunnera* symbiosis. New Phytol 153:297–305

Bockholt R, Scholter-Beck G, Pistorius EK (1996) Construction and partial characterization of an L-amino-acid oxidase-free *Synechococcus* PCC 7942 mutant and localization of the L-amino-acid oxidase in the corresponding wild-type. Biochim Biophys Acta 1307:111–121

Breecker DO, Sharp ZD, McFadden LD (2010) Atmospheric CO2 concentrations during greenhouse climates were similar to those predicted for A.D. 2100. Proc Natl Acad Sci USA 107(2):576–580

Brocks JJ, Pearson A (2005) Building the biomarker tree of life. Rev Mineral Geochem 59:233–258

Brown CM, MacKinnon JD, Cockshutt AM, Villareal TA, Campbell DA (2008) Flux capacities and acclimation cost in *Trichodesmium* from the Gulf of Mexico. Mar Biol 154:413–422

Brummer F, Pfannkunchen M, Bultz A, Hausen T, Thiel V (2008) Light inside sponges. J Exp Mar Biol Ecol 367:61–64

Burns RA, MacDonald CD, McGinn PJ, Campbell DA (2005) Inorganic carbon repletion disrupts photosynthetic acclimation to low temperature in the cyanobacterium *Synechococcus elongatus*. J Phycol 41:322–334

Burns RA, MacKenzie TDB, Campbell DA (2006) Inorganic carbon repletion constrains steady-state light acclimation in the cyanobacterium *Synechococcus elongatus*. J Phycol 42:610–621

Caldeira K, Kasting JF (1992) The life span of the biosphere of the biosphere revisited. Nature 360:721–723

Chen C-TA, Borges AV (2009) Reconciling opposing views on carbon cycling in the coastal ocean: continental shelves as sinks and near-shore ecosystems as sources of atmospheric CO_2. Deep Sea Res II Curr Top Oceanogr 56:578–590

Cole JJ, Caraco NF, Kling GW, Kratz TK (1994) Carbon dioxide supersaturation in the surface waters of lakes. Science 265:1568–1570

Collins S, Bell G (2004) Phenotypic consequences of 1000 generations of selection at elevated CO_2 in a green alga. Nature 431:566–569

Corsetti FA, Awramik SM, Pierce D (2003) A complex microbiota from snowball Earth times: microfossils from the Neoproterozoic Kingston Peak Formation, Death Valley, USA. Proc Natl Acad Sci USA 100:4399–4404

Cowan IR, Lange OL, Green TGA (1992) Carbon dioxide exchange in lichens. Determination of transport and carboxylation characteristics. Planta 187:292–294

Cox GC, Hiller RG, Larkum AWD (1965) An unusual cyanophyta, containing phycourobilin and symbiotic with ascidians and sponges. Mar Biol 89:149–163

Cumino AC, Marcozzi C, Barreiro R, Salerno GL (2007) Carbon cycling in *Anabaena* sp. PCC 7120. Sucrose synthesis in the heterocysts and possible role in nitrogen fixation. Plant Physiol 143:1385–1397

Dittrich M, Obst M (2004) Are picoplankton responsible for calcite precipitation in lakes? Ambio 33:559–564

Dittrich M, Kurz P, Wehrli B (2004) The role of picocyanobacteria in calcite precipitation in an oligotrophic lake. Geomicrobiol J 21:45–53

Dodds WK, Gudder DA, Mollenhauer D (1995) The ecology of *Nostoc*. J Phycol 31:2–18

Doney SC, Tilbrook B, Roy S, Metzl N, Le Quéré C, Hood M, Feely RA, Bakker RA (2008) Surface-ocean CO_2 variability and vulnerability. Deep Sea Res II Top Stud Oceanogr 56:504–511

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO_2 problem. Annu Rev Mar Sci 1:169–192

Droop MR (1974) Heterotrophy of carbon. In: Strewart WDP (ed) Algal physiology and biochemistry. Blackwell Scientific Publications, Oxford, pp 530–559, 989 pp

Duarte CH, Prairie YT, Montes C, Cole JJ, Striegel R, Melack J, Downing JA (2008) CO_2 emissions from saline lakes: a global estimate of a surprisingly large flux. J Geophys Res Biogeosci 113:G0404

Dyer PS (2002) Hydrophobins in the lichen symbiosis. New Phytol 154:1–4

Dyhrman ST, Haley ST (2006) Phosphorus scavenging in the unicellular marine diazotroph *Crocosphaera watsonii*. Appl Environ Microbiol 72:1452–1458

Dyhrman ST, Chappel PD, Halet ST, Moffett JW, Orchard ED, Waterbury JB, Webb EA (2006) Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature 439:68–71

Eisenhut M, Kahlon S, Hasse D, Ewald R, Lieman-Hurwitz J, Oawa T, Wolfgang R, Baume H, Kaplan A, Hagemann M (2006) The plant-like C2 glycolate pathway and the bacteria-like glycerate-pathway cooperate in phosphoglycolate metabolism in cyanobacteria. Plant Physiol 142:333–342

Eisenhut M, Ruth W, Haimovitch M, Bauwe H, Kaplan A, Hagemann M (2008) The photorespiratory glucolate metabolism and might have been conveyed endosymbiotically to plants. Proc Natl Acad Sci 105:17199–17204

Falkowski PG, Raven JA (2007) Aquatic photosynthesis, 2nd edn. Princeton University Press, Princeton, 484 pp

Finkel Z, Finkel ZV, Beardall J, Flynn KJ, Quigg A, Raven JA, Rees TAV (2010) Phytoplankton in a changing world: cell size and elemental stoichiometry. J Plankton Res 32:118–137

Fu FX, Warner ME, Zhan YH, Fen YY, Hutchins DA (2007) Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). J Phycol 43:485–496

Fu FX, Mulholland MR, Garcia NS, Beck A, Bernhardt PW, Warner ME, Sañudo-Wilhelmy SA, Hutchins DA (2008) Interactions between changing pCO_2, N_2 fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera*. Limnol Oceanogr 53:2472–2484

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. Nature 399:541–548

Gadd GM, Raven JA (2010) Geomicrobiology of eukaryotic microorganisms. Geomicrobiol J 27:491–519

Gao KS, Ai HX (2004) Relationship of growth and photosynthesis with colony size in an edible cyanobacterium, Ge-Xian-Mi, *Nostoc* (Cyanophyceae). J Phycol 40:523–526

Gao KS, Yu A (2000) Influence of CO_2, light and watering on growth of *Nostoc flagelliforme* mats. J Appl Phycol 12:185–189

Gao K-S, Zou D (2001) Photosynthetic bicarbonate utilization by a terrestrial cyanobacterium, *Nostoc flagelliforme* (Cyanophyceae). J Phycol 37:768–771

Gattuso J-P, Lavigne H (2009) Technical note: approaches and software tools to investigate the impact of ocean acidification. Biogeosciences 6:2121–2133

Giordano M, Beardall J, Raven JA (2005) CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation and evolution. Annu Rev Plant Biol 56:99–151

Giordano M, Norici A, Ratti S, Raven JA (2008) Role of sulphur for algae: acquisition, metabolism, ecology and evolution. In: Knaff DB, Leustek T (eds) Sulphur metabolism in phototrophic organisms. Springer, Dordrecht, pp 405–433, 516 pp

Gomez-Baena G, Lopez-Lozana A, Gil-Martinez J, Mauel J, Diez J, Candau P, Garcia-Fernandez JM (2008) Glucose uptake and its effect on gene expression in *Prochlorococcus*. PLoS One 3:e3416

Grotzinger JP, Knoll AH (1999) Stromatolites in Precambrian carbonates: evolutionary milestones or evolutionary dipsticks? Annu Rev Earth Planet Sci 27:313–358

Grotzinger JP, Rothman DH (1996) An abiotic model for stromatolite morphogenesis. Nature 383:423–425

Hammer A, Hodgson D-RW, Cann MJ (2006) Regulation of prokaryotic adenylyl cyclases by CO_2. Biochem J 396:215–218

Hansell DA, Kadko D, Bates NR (2004) Degradation of terrigenous organic matter in the Western Arctic Ocean. Science 304:858–861

Hansen PJ, Lundholm N, Rost B (2007) Growth limitation in marine red-tide dinoflagellates: effect of pH versus inorganic carbon availability. Mar Ecol Prog Ser 334:63–71

Hecky RE, Kilham P (1973) Diatoms in alkaline, soda lakes: ecology and geochemical considerations. Limnol Oceanogr 18:53–71
Hellebust JA (1974) Extracellular products. In: Stewart WDP (ed) Algal physiology and biochemistry. Blackwell Scientific Publications, Oxford, pp 838–863, 989 pp
Honegger R (1998) The lichen symbiosis – what is so spectacular about it? Lichenologist 30:193–212
Horne AJ, Goldman CR (1994) Limnology, 2nd edn. McGraw-Hill, New York, 576 pp
Hu HH, Zhou QB (2010) Regulation of inorganic carbon acquisition by nitrogen and phosphorus levels in the *Nannochloropsis* sp. World J Microbiol Biotechnol 26:957–961
Hurd CL, Hepburn CD, Currie KL, Raven JA, Hunter KA (2009) Testing the effects of ocean acidification on algal metabolism: consideration of experimental design. J Phycol 45:1236–1251
Hutchins DA, Fu FX, Zhan Y, Warner ME, Feng Y, Portune K, Bernhardt PW, Mulholland MR (2007) CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry. Limnol Oceanogr 52:1293–1304
Illykchyan IN, Mckay RML, Zehr JP, Dyhrman JT, Bullerjahn GS (2009) Detection and expression of the phosphonate transporter gene in marine and freshwater picocyanobacteria. Environ Microbiol 11:1314–1324
Johnson DT, Wolf-Simon F, Pearson A, Knoll AH (2009) Anoxygenic photosynthesis modulated Proterozoic oxygen and sustained Earth's Middle Age. Proc Natl Acad Sci USA 106:16925–16929
Jones BE, Grant WD, Duckworth AW, Owenson GG (1998) Microbial diversity in soda lakes. Extremophiles 2:191–200
Kah LC, Riding R (2007) Mesoproterozoic carbon dioxide levels inferred from calcified cyanobacteria. Geology 35:799–802
Kaplan A, Reinhold L (1999) CO_2 concentrating mechanisms in photosynthetic microorganisms. Annu Rev Plant Physiol Plant Mol Biol 50:539–559
Kaplan A, Badger MR, Berry JA (1980) Photosynthesis and the intracellular carbon pool in the blue-green alga *Anabaena variabilis*: response to external CO_2 concentrations. Planta 149:219–226
Kelly DP (1971) Autotrophy: concepts of lithotrophic bacteria and their organic metabolism. Annu Rev Microbiol 25:177–210
Kempe S, Kazmierczak J, Landam G, Konuk T, Reimer A, Lipp A (1991) Largest known microbialites discovered in Lake Van, Turkey. Nature 349:605–608
Kneip C, Lockhart P, Voβ C, Maier U-W (2007) Nitrogen fixation in eukaryotes – new models for symbiosis. BMC Evol Biol 7:55
Kneip C, Voβ C, Lockhart PJ, Maier UG (2008) The cyanobacterial endosymbiont of the unicellular alga *Rhopalodia gibba* shows reductive genome evolution. BMC Evol Biol 8:30
Kompantseva UI, Komova AV, Rusnov II, Pimenov NV, Sotokin DY (2009) Primary production of organic matter and phototrophic communities in the soda lakes of the Kolunda Steppe (Altai Krai). Mikrobiologiya 78:703–715
Konishi Y, Prince J, Knott B (2001) The fauna of thrombolytic microbialites, Lake Clifton, Western Australia. Hydrobiologia 457:39–47
Kopp RE, Kirschvink JL, Hilburn IA, Nash CZ (2005) The Palaeoproterozoic snowball Earth: a climatic disaster triggered by the evolution of oxygenic photosynthesis. Proc Natl Acad Sci USA 102:11131–11136
Kosamu IBM, Obst M (2009) The influence of picocyanobacterial photosynthesis on calcite precipitation. Int J Environ Sci Technol 6:557–562
Kranz SA, Sultemeyer D, Richter KU, Rost B (2009) Carbon acquisition by *Trichodesmium*: the effects of pCO_2 and diurnal changes. Limnol Oceanogr 54:548–559
Kranz SA, Levitan O, Richter K-U, Prášil O, Berman-Frank O, Rost B (2010) Combined effects of CO_2 and light on the N_2 fixing cyanobacterium *Trichodesmium* IMS101: physiological responses. Plant Physiol 154:334–345
Kühl M, Chen M, Ralph PJ, Scheiber U, Larkum AWD (2005) A niche for cyanobacteria containing chlorophyll d. Nature 433:820
Lee BD, Apel WA, Waton MR (2004) Screening of cyanobacterial species for calcification. Biotechnol Prog 20:1345–1351
Lemloh M-L, Fremont J, Brümer F, Usher KM (2009) Diversity and abundance of photosynthetic sponges in temperate Western Australia. BMC Ecol 9:4. doi:10.1186/1472-6785-9-4
Levitan O, Rosenberg G, Setlik I, Stelikova E, Grigel J, Klepetar PO, Berman-Frank I (2007) Elevated CO_2 enhances nitrogen fixation and growth in the marine cyanobacterium *Trichodesmium*. Glob Change Biol 13:531–538
Levitan O, Kranz SA, Spungin D, Prášil O, Rost B, Berman-Frank O (2010) Combined effects of CO_2 and light on the N_2 fixing cyanobacterium *Trichodesmium* IMS101: a mechanistic view. Plant Physiol 154:346–356
Li YG, Gao KS (2004) Photosynthetic physiology and growth as a function of colony size in the cyanobacterium *Nostoc sphaeroides*. Eur J Phycol 39:9–15
Lieman-Hurwtiz J, Haimovitch M, Shalev-Malul G, Ishii A, Hihara Y, Gaathon A, Lebendiker M, Kaplan A (2009) A cyanobacterial AbrB-like protein affects the apparent photosynthetic affinity for CO_2 by modulating low CO_2-induced gene expression. Environ Microbiol 11:927–936
Lovelock JE, Whitfield M (1982) Life-span of the biosphere. Nature 296:561–563
Maberly SC (1983) The interdependence of photon irradiance and free carbon dioxide or bicarbonate concentration on the photosynthetic compensation points of freshwater plants. New Phytol 93:1–12
Maberly SC (1996) Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. Freshw Biol 35:579–598
Maberly SC, Spence DHN (1983) Photosynthetic inorganic carbon use by freshwater plants. J Ecol 71:705–724
Maberly SC, Ball LA, Raven JA, Sültemeyer D (2009) Inorganic carbon acquisition by chrysophytes. J Phycol 45:1052–1061
MacKenzie TDB, Campbell DA (2005) Cyanobacterial acclimation to rapidly fluctuating light is constrained by inorganic carbon status. J Phycol 41:801–811
MacKenzie TDB, Burns RA, Campbell DA (2004) Carbon status constrains light acclimation in the cyanobacterium *Synechococcus elongatus*. Plant Physiol 136:3301–3312
MacKenzie TDB, Johnson JM, Campbell DA (2005a) Dynamics of fluxes through photosynthetic complexes in response to changing light and inorganic carbon accumulation in *Synechococcus elongantus*. Photosynth Res 85:341–357
MacKenzie TDB, Johnson JM, Cockshutt AM, Burns RA, Campbell DA (2005b) Large reallocation of carbon, nitrogen and photosynthetic reductant among phycobilisomes, photosystems and Rubisco during light acclimation in *Synecchococcus elongatus* strain PCC7942 are constrained in cells under low environmental inorganic carbon. Arch Microbiol 183:192–202
Maguas C, Griffiths H, Broadmeadow H (1995) Gas-exchange and carbon-isotope discrimination in lichens – evidence for interactions between CO_2-concentrating mechanisms and diffusion limitation. Planta 196:95–102
Martiny AC, Kathuris S, Berube PM (2009) Widespread metabolic potential for nitrite and nitrate assimilation among *Prochloroccus* genotypes. Proc Natl Acad Sci 106:10787–10792
Mary I, Garkzarek L, Tarran GA, Koloirat C, Terry MJ, Scanlan DJ, Burkhill PH, Zubkov MV (2008) Diel periodicity in amino acid uptake by *Prochlorococcus*. Environ Microbiol 10:2124–2151
Mcloughlin N, Wilson LA, Brasier MD (2008) Growth of synthetic stromatolites and wrinkle structures in the absence of microbes – implications for the early fossil record. Geobiology 6:95–105
Melack JM (1979) Photosynthesis and growth of *Spirulina platensis* (Cyanophyta) in an equatorial lake (Lake Smibi, Kenya). Limnol Oceanogr 24:753–760

Melack JM, Kilham P (1974) Photosynthetic rates of phytoplankton in East African alkaline, saline lakes. Limnol Oceanogr 19:743–755

Meyer M, Seibt U, Griffiths H (2008) To concentrate or ventilate? Carbon acquisition, isotope discrimination and physiological ecology of early land plant life forms. Philos Trans R Soc B 363:2767–2778

Mulholland MR, Glibert PM, Berg GM, van Heukelem L, Pantoja S, Lee C (1998) Extracellular amino acid oxidation by microplankton: a cross-ecosystem comparison. Aquat Microbiol Ecol 15:141–152

Mulkadjinian AY, Koonin EY, Makarova KS, Mekhedova KS, Sorokin A, Wolf YI, Dufresne A, Partensky F, Burd H, Kaznadey D, Haslkorn R, Galperin MY (2006) The cyanobacterial genome core and the origin of photosynthesis. Proc Natl Acad Sci 103:13126–13131

Obst M, Wewhrli B, Dittrich M (2009) $CaCO_3$ nucleation by cyanobacteria: laboratory evidence for a passive, surface-induced mechanism. Geobiology 3:324–347

Oliveri E, Neri R, Bellanca A, Riding R (2010) Carbonate stromatolites from a Messinian hypersaline setting in the Caltanissetta Basin, Sicily: petrographic evidence of microbial activity and related stable isotope and rare earth element signatures. Sedimentology 57:142–161

Palinska KA, Laloui W, Bédu S, Loiseaux-de Goër S, Castets AM, Tippka R, Tandeau de Marsac N (2002) The signal transducer P_{II} and bicarbonate acquisition in *Prochlorococcus marinus* PCC 9511, a marine cyanobacterium naturally deficient in nitrate and nitrite assimilation. Microbiology 148:2405–2412

Palmqvist K (1993) Photosynthetic CO_2-use efficiency in lichens and their isolated photobionts: the possible role of a CO_2-concentrating mechanism. Planta 191:48–56

Palmqvist K (2000) Carbon economy in lichens. New Phytol 148:11–36

Palmqvist K, Maguas C, Badger MR, Griffiths H (1994) Assimilation, accumulation and isotope discrimination of inorganic carbon in lichens: further evidence for the operation of a CO_2 concentrating mechanism in cyanobacterial lichens. Cryptogam Bot 4:218–226

Perry RS, Mcloughlin N, Lyme BY, Sephton MA, Oliver JD, Perry CC, Campbell K, Engel MH, Farmer JD, Barier MD, Staley JT (2007) Defining biominerals and organominerals: direct and indirect indicators of life. Sediment Geol 201:157–179

Poza-Carrión C, Fernández-Valiente E, Pi as FF, Leganés F (2001) Acclimation of photosynthetic pigments and photosynthesis of the cyanobacterium *Nostoc* sp. Strain UAM206 to combined fluctuating irradiance, pH, and inorganic carbon availability. J Plant Physiol 158:1455–1461

Price GD, Badger MR, Woodger FJ, Long BM (2008) Advances in understanding the cyanobacterial CO_2-concentrating-mechanism (CCM): functional components, C_i transporters, diversity, generic regulation and prospects for engineering into plants. J Exp Bot 59:1441–1461

Qiu BS, Gao KS (2001) Photosynthetic characteristics of the terrestrial blue-green alga, *Nostoc flagelliforme*. Eur J Phycol 36:147–156

Qiu BS, Gao K (2002a) Daily production and photosynthetic characteristics of *Nostoc flagelliforme* grown under ambient and elevated CO_2 conditions. J Appl Phycol 14:77–83

Qiu BS, Gao K (2002b) Effect of CO_2 enrichment on the bloom-forming cyanobacterial *Microcystis aeruginosa* (Cyanophyceae): physiological responses and relationships with the availability of dissolved inorganic carbon. J Phycol 38:721–729

Rai AN, Söderböek E, Bergman B (2000) Cyanobacterium-plant symbioses. New Phytol 147:449–481

Rai AN, Bergman B, Rasmussen U (eds) (2002) Cyanobacteria in symbiosis. Springer, Dordrecht

Ramos JBE, Biswas H, Schulz K, LaRoche J, Riebesell U (2007) Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*. Glob Biogeochem Cycles 21(2):GB2028

Ran L, Larrson J, Vigil-Stenman T, Nylander JAA, Ininbergs K, Zheng W-W, Lapidus A, Lowry S, Haselkorn R, Bergman B (2010) Genome erosion in a vertically-transmitted endosymbiotic multicellular cyanobacterium. PLoS One 5(7):e11486. doi:10.371/journal.pone/0011486

Rasmussen B, Fletcher IR, Brocks JJ, Kilburn MR (2008) Reassessing the first appearance of eukaryotes and cyanobacteria. Nature 455:1101–1104

Raun AL, Borum J, Sand-Jensen K (2009) Active accumulation of internal DIC pools reduces transport limitation in large colonies of *Nostoc pruniforme*. Aquat Biol 5:23–29

Raven JA (1984a) Energetics and transport in aquatic plants. Alan R Liss, New York, 587 pp

Raven JA (1984b) A cost-benefit analysis of photon absorption by photosynthetic unicells. New Phytol 98:593–625

Raven JA (1986) Evolution of plant life forms. In: Givnish T (ed) On the economy of plant form and function. Cambridge University Press, New York, pp 421–492, 717 pp

Raven JA (1993) Energy and nutrient acquisition by autotrophic symbioses and their asymbiotic ancestors. Symbiosis 14:33–60

Raven JA (1997) The role of marine biota in the evolution of terrestrial biota: gases and genes. Biogeochemistry 39:139–164

Raven JA (1999) The flagellate condition. In: Leadbeater BSC, Green JC (eds) The flagellates: unity, diversity and evolution. Taylor and Francis, London, pp 27–48

Raven JA (2002) Evolution of cyanobacterial symbioses. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 329–346, 355 pp

Raven JA (2003) Inorganic carbon concentrating mechanisms in relation to the biology of algae. Photosynth Res 77:155–171

Raven JA (2006) Sensing inorganic carbon: CO_2 and HCO_3^-. Biochem J 396:e5–e7

Raven JA (2009) Contributions of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments. Aquat Microb Ecol 56:177–192

Raven JA, Falkowski PG (1999) Oceanic sinks for atmospheric CO_2. Plant Cell Environ 22:741–755

Raven JA, Giordano M (2009) Biomineralization by photosynthetic organisms: evidence of coevolution of the organisms and their environment. Geobiology 7:140–154

Raven JA, Knoll AH (2010) Non-skeletal biomineralization by eukaryotes: matters of moment and gravity. Geomicrobiol J 27:572–584

Raven JA, Larkum AWD (2007) Are there ecological implications for the proposed energetic restrictions on photosynthetic oxygen evolution at high oxygen concentrations. Photosynth Res 94:31–42

Raven JA, Samuelsson G (1998) Ecophysiology of *Fucus vesiculosus* L. close to its northern limit in the Gulf of Bothnia. Bot Mar 31:399–410

Raven JA, Johnston AM, Handley LL, McInroy SG (1990) Transport and assimilation of inorganic carbon by *Lichina pygmaea* under emersed and submersed conditions. New Phytol 114:407–417

Raven JA, Kübler JE, Beardall J (2000) Put out the light, and then put out the light. J Mar Biol Assoc UK 80:1–25

Raven JA, Johnston AM, Kűbler JE, Korb RE, McInroy SG, Handley LL, Scrimgeour CM, Walker DI, Beardall J, Vanderklift M, Fredricksen J, Dunton KH (2002) Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. Funct Plant Biol 29(2–3):355–378

Raven JA, Brown K, Mackay M, Beardall J, Giordano M, Granum E, Leegood RC, Kilminster K, Walker DI (2005) Iron, nitrogen, phosphorus and zinc cycling and consequences for primary productivity of the oceans. In: Gadd GM, Semple KT, Lappin-Scott HM (eds) Micro-organisms and earth systems: advances in geobiology, Society for general microbiology symposium 65. Cambridge University Press, Cambridge, pp 247–272

Raven JA, Cockell CS, De La Rocha CL (2008a) The evolution of inorganic carbon concentrating mechanisms in photosynthesis. Philos Trans R Soc Lond B 363:2641–2650

Raven JA, Giordano M, Beardall J (2008b) Insights into the evolution of CCMs from comparison with other resource acquisition and assimilation processes. Physiol Plant 133:4–14

Raven JA, Beardall J, Giordano M, Maberly SC (2011) Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. Photosynth Res 109(1–3):281–296

Revsbeck NP, Jørgensen BB, Brix O (1981) Primary production of microalgae in sediments measured by oxygen microprofle, $H^{14}CO_3^-$ fixation, and oxygen exchange methods. Limnol Oceanogr 26:717–730

Riding R (2006) Cyanobacterial calcification, carbon dioxide concentration mechanisms, and Proterozoic-Cambrian changes in atmospheric composition. Geobiology 4:299–316

Riding R (2008) Abiogenic, microbial and hybrid authigenic carbonate crusts: componenst of Precambrian stromatolites. Geol Croat 61:73–103

Riding R (2009) An atmospheric stimulus for cyanobacterial-bioinduced calcification ca. 350 million years ago? Palaios 24:685–696

Rittenberg SC (1972) The obligate autotroph – the demise of a concept. Anthonie van Leeuwenhoek 38:457–478

Rost B, Zondervan I, Wolf-Gladrow D (2008) Sensitivity of phytoplankton to future changes in ocean acidification: current knowledge, contradictions and research directions. Mar Ecol Prog Ser 373:227–237

Rothschild LJ, Mancinelli RL (1990) Model of carbon fixation in microbial mats from 3,500 Myr ago to the present. Nature 345:710–712

Sand-Jensen K (2009) Fascinating adaptation of plants in *Lobelia* lakes. Sven Bot Tidskr 103:174–182

Sand-Jensen J, Pedersen MF (1994) Photosynthesis by symbiotic algae in the freshwater sponge, *Spongilla lacustris*. Limnol Oceanogr 39:551–561

Sand-Jensen K, Raun AL, Borum J (2009) Metabolism and resources of spherical colonies of *Nostoc zetterstedtii*. Limnol Oceanogr 54:1282–1291

Schreik S, Rückert C, Staiger D, Pistorius EK, Michel K-P (2007) Bioinformatic evaluation of L-arginine catabolic pathways in 24 cyanobacteria and transcriptional analysis of genes encoding enzymes of L-arginine catabolism in the cyanobacterium *Synechocystis* sp. PCC 6803. BMC Genomics 8:437. doi:10.1186/1471.2164-8-437

Schulz KG, Barcelos e Ramos J, Zeebe RE, Riebesell U (2009) CO_2 perturbation experiments: similarities and difference between dissolved inorganic carbon and total alkalinity manipulations. Biogeosciences 6:2145–2153

Scott KM, Henn-Sax M, Harmer TL, Longo DL, Frame CH, Cavanaugh CM (2007) Kinetic isotope effect and biochemical characterization of the form IA RubisCO from the marine cyanobacterium *Prochlorococcus marinus* MIT9313. Limnol Oceanogr 52:2199–2204

Sherrat TN, Wilkinson DM (2009) Big questions in ecology and evolution. Oxford University Press, Oxford, 297 pp

Shi T, Falkowski PG (2008) Genome evolution in cyanobacteria. The stable core and the variable shell. Proc Natl Acad Sci USA 105:2510–2515

Shi D, Xu Y, Morel FMM (2009) Effects of pH/pCO_2 control methods on medium chemistry and phytoplankton growth. Biogeosciences 6:1199–1207

Smith EC, Griffiths H (1998) Intraspecific variations in the photosynthetic responses in cyanobacterial lichens from contrasting habitats. New Phytol 138:213–224

Sobek S, Algeston G, Bergstrom AK, Jansson M, Tranvick LJ (2005a) The catchment and climate regulation of pCO_2 in boreal lakes. Glob Change Biol 9:630–641

Sobek S, Tranvick LJ, Cole JJ (2005b) Temperature independence of carbon dioxide supersaturation in global lakes. Glob Biogeochem Cycles 19:GB2003

Song YF, Qiu BS (2007) The CO_2-concentrating mechanism in the bloom-forming cyanobacterium *Microcystis aeruginosa* (Cyanophyceae) and effects of UVB radiation on its operation. J Phycol 43:957–964

Steinberg NA, Meeks JC (1989) Photosynthetic CO_2 fixation and ribulose bisphosphate carboxylaase/oxygenase activity of *Nostoc* sp. Strain UCD 7801 in symbiotic association with *Anthoceros punctatum*. J Bacteriol 174:6227–6233

Steinberg CEW, Schäfer H, Beisker W (1998) Do acid-tolerant cyanobacteria exist? Acta Hydrochim Hydrobiol 26:13–19

Takahashi T, Sutherland SC, Wanninkhof R, Eweeney C, Feely RA, Chipman DW, Hales B, Friederich G, Chavez F, Sabine C, Watson A, Bakker DC-E, Schuster U, Metzl N, Yoshikawa-Inoue T, Ishii M, Midorikawa T, Nojiri Y, Körtzinger A, Steinhoff T, Hoppema M, Olafsson J, Arnarson TA, Tuilbrook B, Johannessen T, Olsen A, Bellerby R, Won CS, Bates NR, de Baar HJW (2009) Climatological mean and decadal change in surface ocean pCO_2, and net sea-air CO_2 flux over the global oceans. Deep Sea Res II Top Stud Oceanogr 56:554–577

Talling JF (1965) The photosynthetic activity of phytoplankton in East African lakes. Int Rev Gesamt Hydrobiol 50:1–32

Talling JF, Talling IB (1965) The chemical composition of African lakes and waters. Int Rev Gesamt Hydrobiol 50:421–463

Talling JF, Wood RB, Prosser MV, Baxter RM (1973) The upper limit of photosynthetic productivity of phytoplankton: evidence from Ethiopean soda lakes. Freshw Biol 3:53–76

Taylor MW, Hill RT, Piel J, Thacker RW, Hertschel U (2007) Soaking it up: the complex lives of marine sponges and their microbial associates. ISME J 1:187–190

Tcherkez GG, Farquhar GD, Andrews TJ (2006) Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimised. Proc Natl Acad Sci USA 103:7246–7251

Thomas DJ, Sullivan SI, Price AL, Zimmerman SM (2005) Common freshwater algae grow in 100% CO_2. Astrobiology 5:66–74

Tomitani A, Knoll AH, Cavanaugh CM, Ohno T (2006) The evolutionary diversity of cyanobacteria: molecular-phylogenetic perspectives. Proc Natl Acad Sci USA 103:5442–5447

Tredici MR, Maiheri MC, Giovanetti L, de Philippis R, Vincenzini M (1988) Heterotrophic metabolism and diazotrophic growth of *Nostoc* sp. from *Cycas circinalis*. Plant Soil 110:199–206

Ungerer JL, Pratte BS, Thiel T (2008) Regulation of fructose transport and its effects on fructose toxicity in *Anabaena* spp. J Bacteriol 190:8115–8125

Usher KM, Bergman B, Raven JA (2007) Exploring cyanobacterial mutualisms. Annu Rev Ecol Evol Syst 38:255–273

Vuorio K, Meili M, Sarvala J (2009) Natural isotope composition of carbon ($\delta^{13}C$) correlates with colony size in the planktonic cyanobacterium *Gloeotrichia echinulata*. Limnol Oceanogr 54:925–929

Wawrick B, Callagan AK, Bronck DA (2009) Use of inorganic and organic nitrogen by *Synechococcus* spp. and diatoms on the West Florida Shelf as measured using stable isotope probing. Appl Environ Microbiol 75:6662–6670

Whitton BA, Al-Shehri AM, Ellwood NTW, Turner BJ (2005) Ecological aspccts of phosphatise activity in cyanobacteria, eukaryotic algae and bryophytes. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. Commonwealth Agricultural Bureau, Wallingford, pp 205–241, 399 pp

Wood AP, Aurikko JP, Kelly DP (2004) A challenge for 21st century molecular biology and biochemistry: what are the causes of autotrophy and methanotrophy? FEMS Microbiol Lett 28:335–352

Woodger FJ, Badger MR, Price GD (2005) Sensing of inorganic carbon limitation in *Synechococcus* PCC7942 is correlated with the size of the inorganic carbon pool and involves oxygen. Plant Physiol 139:698–710

Wouters J, Raven JA, Minnhagen S, Graneli E, Janson S (2009) The luggage hypothesis: comparison of two phototrophic hosts with nitrogen-fixing cyanobacteria and implications on analogous life histories for kleptoplastids/secondary symbiosis in dinoflagellates. Symbiosis 49:61–70

Xu Z, Gao KS (2009) Impacts of UV radiation on growth and photosynthetic carbon assimilation in *Gracilaria lemanaeiformis* (Rhodophyta) under phosphorus-limited and replete conditions. Funct Plant Biol 36:1057–1064

Xu T, Song L-R (2007) Studies on the utility of inorganic carbon in three strains of *Microcystis aeruginosa*. Acta Hydrol Sin 31:245–250

Zeebe RE, Wolf-Gladrow D (2001) CO_2 in seawater: equilibrium, kinetics. Isotopes, Elsevier, 346 pp

Zehr JP, Bench SR, Carter BJ, Hewson I, Niazi F, Shi T, Tripp HJ, Affourtit JP (2008) Globally distributed uncultivated oceanic N_2-fixing cyanobacteria lack oxygenic photosystem II. Science 322:1110–1112

Zhang C-C, Jeanjean R, Joset F (1998) Obligate phototrophy in cyanobacteria: more than a lack of sugar transport. FEMS Microbiol Lett 161:285–292

Zubkov MV (2009) Photoheterotrophy in marine prokaryotes. J Plankton Res 31:933–938

Note added in Proof

The paper of Roberts et al. (2012) on the isolation and characterisation of carboxysomes from the α-cyanobacterium *Prochlorococcus* is relevant to Section 17.2.2. Roberts et al. (2012) isolated pure carboxysomes from *Prochlorococcus* MRD4, and found a novel shell protein CsoS1D which is shared with other α-cyanobacteria. The function of CsoS1D is suggested to relate to metabolite transfer across the carboxysome shell (Roberts et al. 2012); how this relates to the ecology of these marine cyanobacteria requires further investigation.

Work on the tricarboxylic acid cycle in cyanobacteria (Zhang and Bryant 2011) is relevant to the nature of obligate photolithotrophy in cyanobacteria (Section 17.3.2). An attractive hypothesis to explain the obligate photolithotrophy in many cyanobacteria is that, because they lack 2-oxoglutarate dehydrogenase, they have an incomplete tricarboxylic acid cycle and so have problems with the energetics of chemo-organotrophic and, perhaps, photo-organotrophic growth (Zhang and Bryant 2011). However, Zhang and Bryant (2011) found that the β-cyanobacteria, but not α-cyanobacteria, which they examined possess two enzymes (2-oxoglutarate decarboxylase and succinic semialdehyde dehydrogenase) which can substitute for 2-oxoglutarate dehydrogenase. The results of Zhang and Roberts (2011) have important implications for the functional basis of obligate photolithotrophy in cyanobacteria. The view that obligate photolithotrophy in cyanobacteria is a result of an incomplete tricarboxylic acid cycle due to the absence of 2-oxoglutarate dehydrogenase has been shown to only apply to *Prochlorococcus* and marine *Synechococcus* (Zhang and Bryant 2011). For all of other cyanobacteria two novel enzymes, 2-oxoglutarate decarboxylase and succinic semialdehyde dehydrogenase, catalyse the tricarboxylic acid cycle reactions, which are usually catalysed by 2-oxoglutarate dehydrogenase and succinyl-CoA synthetase (Zhang and Bryant 2011). These important results mean that the functional basis for obligate photolithotrophy in cyanobacteria other than *Prochlorococcus* and marine *Synechococcus* must be sought elsewhere than in an incomplete tricarboxylic acid cycle.

Raven et al. (2012) consider the evolution of inorganic carbon acquisition in cyanobacteria (and algae) in relation to the origins of the components of the metabolic pathways involved and the variations in atmospheric composition over the last 2.4 billion years (Section 17.5), emphasising the recent evidence for the freshwater origin of cyanobacteria (Sánchez-Baracaldo et al. 2005, Blank and Sánchez-Baracaldo 2010).

References

Blank CE, Sánchez-Baracaldo P (2010) Timing of morphological and ecological innovations in the cyanobacteria – a key to understanding the rise of atmospheric oxygen. Geobiol 8:1–23

Raven JA, Giordano M, Beardall J, Maberly SC (2012) Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. Phil Trans R Soc B 367:493–507

Roberts EW, Cai F, Kerfeld CA, Cannon GC, Heinhorst S (2012) Isolation and characterisation of the *Prochlorococcus* carboxysome reveal the presence of the novel shell protein CsoS1D. J Bacteriol 194:787–795

Sánchez-Baracaldo P, Hayes PK, Blank CE (2005) Morphological and habitat evolution in the Cyanobacteria using a compartmentalization approach. Geobiol 3:145–165

Zhang S, Bryant DA (2011) The tricarboxylic acid cycle in cyanobacteria. Science 334:1551–1553

Extracellular Matrix (ECM)

18

Richard F. Helm and Malcolm Potts

Contents

R.F. Helm (✉)
Department of Biochemistry, Life Sciences I, Virginia Tech, Blacksburg, VA 24061, USA
e-mail: helmrf@vt.edu

M. Potts
Department of Biological and Environmental Sciences, College of Arts and Sciences,, Qatar University, POB 2713, Doha, Qatar
e-mail: malcolm.p@qu.edu.qa

Summary

The region of space at the periphery of cyanobacterial cells is the interface between the environment and intracellular processes. This metaspace may include a structure appressed to the outer wall and membrane, such as an extracellular polysaccharide (EPS), a structural and/or physiological discontinuity modulating metabolite flow, as well as a temporal flux that accompanies stress or cell division. The functional framework within this region is designed to recognize environmental perturbations and relay physical and biochemical information to the cell interior, and perhaps to the cell community, for the appropriate physiological response. Communication between the environment and the cells is thus initiated within this extracellular milieu, which is therefore an important spatial domain in cyanobacteria. The ECM of cyanobacterial cells is multifaceted. It is not only a complex and dynamic mixture of polysaccharides, proteins, cell remnants and lower molecular weight secondary metabolites, but a hyperspace that tunes seasonal as well as short-term stochastic modulations in environmental conditions. Such stresses result in changes in both the composition and organization of the matrix as cyanobacterial cells adjust to the environmental perturbations. This chapter provides a critical appraisal of the ecology and evolution of the cyanobacterial ECM compared with other prokaryotes. Emphasis is placed on how little is understood about this "occupied space" and several hypotheses and examples are presented in an effort to promote additional investigations of this oft-ignored interface.

18.1 Context of the ECM

The diversity of structural features of cyanobacterial cells and colonies emphasizes the complexity of the ECM and raise important questions about its functions and roles.

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18.1.1 ECM – What's in a Name?

A cursory perusal of any text in eukaryotic cell biology that deals with higher forms (Alberts et al. 2008) reveals the primary conceptual importance of the extracellular matrix (the ECM). Here ECM is equated generally with the non-cellular or "extracellular" part of a cell's environment that provides structural support as well as a myriad of other (somewhat less-defined) functions. The term matrix is very much emphasized as, or conceived as, a palpable, physical entity. With the advent of studies on bacterial biofilms (Stoodley et al. 2002; Costerton 2004; Stewart and Franklin 2008) the use of the term ECM in the context of prokaryotes came into use. Very soon, the term ECM became synonymous with EPS (extracellular polysaccharide) in bacterial colonies and populations. While one component of the cyanobacterial ECM is certainly attributed to EPS, the two should not be considered as being equivalent.

The etymology of the word "matrix" has its origins in the mid-sixteenth century deriving from "mother", "maternal" and "womb." One definition is: "A place or medium in which something is originated, produced, or developed; the environment in which a particular activity or process begins; a point of origin and growth" (OED 2009). The use of the word "environment" seems particularly appropriate here since it negates the concept of matrix as a solely tangible physical compartment. Rather, it emphasizes a zone of functionality; admittedly a more difficult concept to grasp and one that has, in addition, temporal attributes.

18.1.2 Old and New ECMs

Forms attributed to ancestral cyanobacteria had discontinuities at their cell surfaces that were sufficiently durable and conspicuous as to leave permanent traces in the fossil record (Chap. 2). Superficially, these structures seem comparable with the extracellular investments of contemporary cyanobacteria; has any functionality been retained to the present day?

In the classical taxonomic literature (Geitler 1932), much emphasis is placed upon the presence, absence, form, colour, texture, extent and development of a plethora of extracellular investments referred to variously as, in German for example, Scheide (meaning sheath, scabbard, border), as well as slimes, glycocalyces, and capsules. Less attention was paid to the complexity of these structures during the transition to the taxonomy *sensu* Rippka and co-workers (Rippka et al. 1979) based on axenic cultures. A primary reason for this was the common loss of interesting structural characteristics during prolonged laboratory culture. Nevertheless, "sheath absent" and "sheath present" are retained as determinative characters (Rippka et al. 1979), but one must question

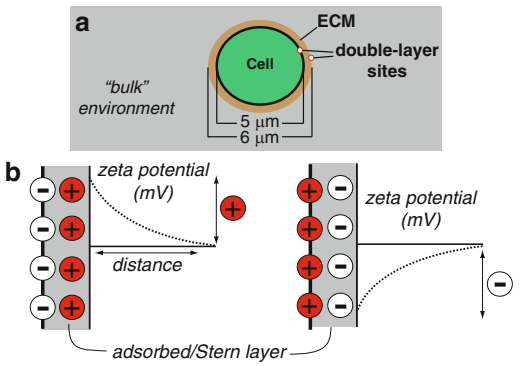

Fig. 18.1 Cells and cellular interfaces: (**a**) A unicellular cyanobacterium, depicted as a spherical cell, occupies a much larger space when producing an ECM. The outer membrane of the cell as well as the exterior surface of the ECM will have electrical double layers, the charge and magnitude of which will be dependent on cellular and bulk environmental conditions. (**b**) Depiction of electrical double layers. The outer membrane and the distal ECM face will have adsorbed counterions that provide a charge differential between the adsorbed layer and the environment. The charge will dissipate rapidly as the distance from the surface increases.

do these equate at the functional and conceptual level, respectively, with "ECM absent" or "ECM present?" For example, do the important members of marine picoplankton (Scanlan et al. 2009) of the genus *Synechococcus* (Section I, "sheath absent"; Chap. 20) have no extracellular matrix in the context of absence of a functional interface (EPS, sheath) with their environment? We think not. The concept of "extracellular" (EC) is easy to accommodate, but "matrix" conjures the sense of a physical scaffold, just one more layer of the cell, with no demarcation regarding functionality. Milieu (environment), i.e. extracellular milieu (ECM), seems a more appropriate term, but it may be harder to adopt at this point. For the rest of this discussion we use the term ECM loosely as a structural, functional and temporal region of ill-defined properties.

18.1.3 Biophysical Considerations

Consider the ECM of an ensheathed unicellular cyanobacterium with a cylindrical to ovoid cell of 5 µm diameter that reproduces by binary transverse fission e.g. *Gloeothece*. In reality, such an ovoid has a quadratic surface that may be somewhat prolate (elongated) or oblate (flattened). However, for the purposes of this first example consider the simplest scenario where the cell is a sphere (Fig. 18.1a). In this case, the extracellular compartment would typically add an additional 0.5 µm thickness to give a total diameter of 6.0 µm. The volume of the ECM is 47.6 μm^3, representing 42% of the total three-dimensional space occupied by the individual cell (ball). This is a substantial volume in comparison to that of the cell interior. Furthermore, addition of this zone to an

otherwise "naked" cell increases the available surface area by 44% (78.6–113 μm^2). If one considers that such a cell is tumbling through a water column, then this can be considered a starting point to consider how that cell interacts either with molecules undergoing molecular diffusion to or from the surface of the ECM, or with objects on the size scale of other cells ("self" or epiphytes), cyanophages or solid surfaces.

It is important to make the distinction between the functional ball of the cell, the space extending out from the cell, and the extreme distal face of the ECM in contact with the environment. Each of these boundaries will have electrical double layers of ill-defined charges and thicknesses (Fig. 18.1b). The charge difference between the surface and bulk fluid is termed the zeta potential, and has been measured in a limited number of cyanobacteria (Dittrich and Sibler 2005; Martinez et al. 2008, 2010). These charge states must be accommodated when posing questions and hypotheses about the structure and function of the cyanobacterial ECM. In addition, one must consider if there are regions of temporal transfer hyperactivity at the outer surface of the ECM. In other words, is the three-dimensional ECM (essentially a hollow sphere of finite thickness possessing an electrical double layer) homogenous? What controls determine "departure" from the junction of the distal surface of the outer cell membrane and proximal face of the ECM, "travel" in the ECM, and "arrival" at the distal extremity of the ECM (bulk fluid); and *vice versa*? In this example the cell's environment was aqueous. What if the environment is aerophytic with sporadic hydration? This will surely increase the complexity of the ECM regiochemical dynamics.

To further develop the context of the ECM, consider an example where an ensheathed cell is spherical, but divides in two or three successive planes at right angles to one another. This is to emphasize that unless one invokes synchronization, and we are not ruling that out, the cells may be at different stages of division at any point in time when they may be forced to undergo metabolic arrest. Now, consider many tens, hundreds, thousands, of these cells, depending on the maturity of the colony, encased (immobilized?) in a second ECM2; essentially a multicellular aggregate. This, in fact, is the situation for *Gloeocapsa* cf *sanguinea* growing on roof shingle (Fig. 18.2). In simplistic terms, one can consider the cells as particles within the ECM2; the system is colloidal, with the ionic strength of the bulk fluid influencing the magnitude and extent of the electrical double layers. Colonies of *Gloeocapsa*, with a range of diameters, become stacked and distributed over the substrate, where they appear as associated spheroidal colonies when viewed in the low power light microscope (Fig. 18.2e). This provides a further dimension to the ECM (*i.e.* ECM3). Consider one colony of 500 μm diameter. If only 1% of the three-dimensional space of ECM2 were available for occupancy, approximately 5,700 cells (3 μm radius) would be encased. If 50% of the space was comprised of cells, then 290,000 could be accommodated.

One reason to consider these hypothetical numbers is that microscopic examination of such colonies, and typically many other types collected from field settings *e.g.* marine *Gomphosphaeria* spp., reveals an apparently ordered geometric distribution of the particles (cells) where no two appear to be in direct contact with another. How could this occur since it seems a violation of thermodynamics? Model experiments with different sized spheres, suspended in water, identified the depletion force, which is entropic in nature and can lead to such ordering in colloidal systems (Cates et al. 2010; Marenduzzo et al. 2006). Such experiments in condensed matter physics may shed much light on the dynamics of the ECM in cyanobacterial communities. Also, application of so-called Voronoi tessellation analysis may be informative (Kumar and Kumaran 2005; Rycroft et al. 2006). In such an analysis one can consider the colony (big ball ECM2) as a system of monodisperse spheres (cells ECM1). The centre of each cell defines a unique set of points within the space of the colony and the resulting Voronoi tessellation is used to study the packing properties of the colony through computer simulation. If the monodisperse cells are non-overlapping, as they are in the *Gloeocapsa* colony, then each sphere (cell with its ECM1) is completely contained within its Voronoi "cell" (Voronoi terminology "cell" is tantamount equivalent to "zone"). Such a "zone" has physical attributes that may be more representative, conceptually, of the ECM in the way that we envisage it to be. The "flow" of cells in this colloidal *Gloeocapsa* system may be slow, in view of the viscosity of the ECMs, but one must emphasize that such flow is still subject to the effects of gravity.

At this point, there has been no discussion of how the *Gloeocapsa* system (with its ECM1 and ECM2, distinct structures, and the additional physical dimension of colony stacking giving ECM3), responds to environmental perturbation. Removal of water from this system through desiccation leads to time-dependent shrinkage of the ECMs and cells, loss of structure, the geometry and packing of the system collapses and cells are now in close contact. This is not unexpected considering the principle role of water. What is confounding however, is that upon subsequent rehydration of the colony, all of the geometry, spatial structure and cell distribution is recovered.

The complexity of this seemingly nondescript roof film is emphasized by the regular three-dimensional distribution of cells within spheres of ECM2 (Fig. 18.2), raising the same types of questions as formulated by Schaudinn et al. (Schaudinn et al. 2007) with regard to the presence of "geometric order" in biofilms. How can the three-dimensional distribution of cells within an aerophytic community biofilm (ACB) be maintained during multiple cycles

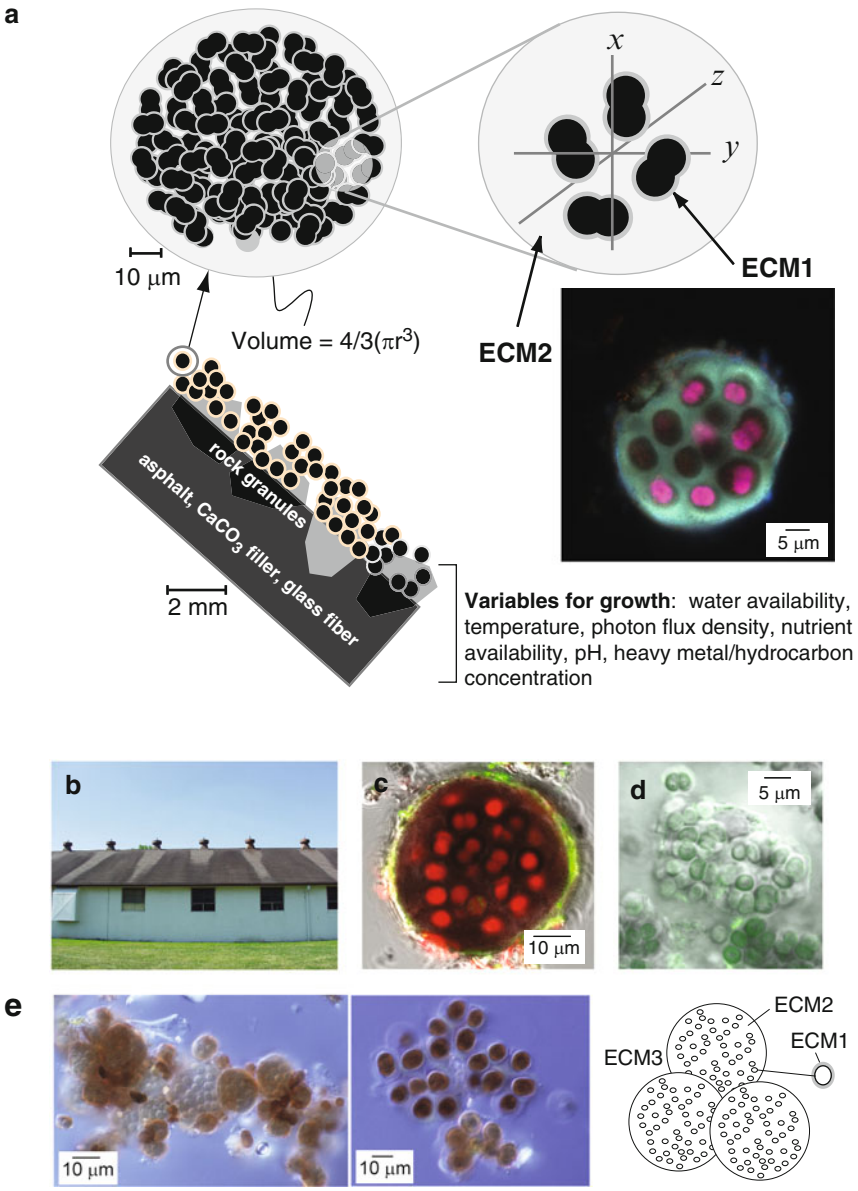

Fig. 18.2 Essential features of the *Gloeocapsa* ECM: (**a**) Roof shingle contains an asphalt base, calcium carbonate filler, and glass fiber, with rock granules embedded in the matrix at the time of manufacture. Colonies of *Gloeocapsa* grow on, around, and between the rock granules, as well as on the asphalt itself. Single colonies have the shape of spheres when hydrated, and contain numerous cells distributed within a polysaccharide matrix (EPS) infiltrated with UV-absorbing pigments, proteins, metabolites and potentially nucleic acids. Combined, this region is referred to as the outer extracellular matrix (ECM2). Note that when hydrated each cell is also bound by a thin, inner ECM (ECM1). (**b**) Abundance of the biofilm can be judged from a comparison of mature community and regions where growth is inhibited by the leaching of metals from roof ducts. (**c**) In these hydrated colonies, no one dividing cell comes into contact with its neighbors; the cells are equally distributed within the spherical, 3-dimensional ECM with a clearly defined distal face. During removal of water and desiccation the outer ECM collapses so that cells either make contact or, remain separated by a biophysical glass with a thickness on the nanometer scale. Upon rehydration, the spherical distribution of cells within ECM is re-instated. (**d**) Laboratory planktonic growth results in a more crowded and less organized ECM-encased community. (**e**) Associations of individual colonies leads to the development of ECM3.

of desiccation (shrinkage and thus cell-cell contacts), subsequent rehydration, heating and freezing? Currently it is thought that ECM provides this capability, but we have no plausible explanation for the underlying controls that would permit this.

The last two examples consider cyanobacteria of Section IV (filamentous heterocystous cyanobacteria that divide in only one plane (Rippka et al. 1979)); specifically *Nostoc commune* and forms where the trichome is tapered and a hair is formed (Chap. 22).

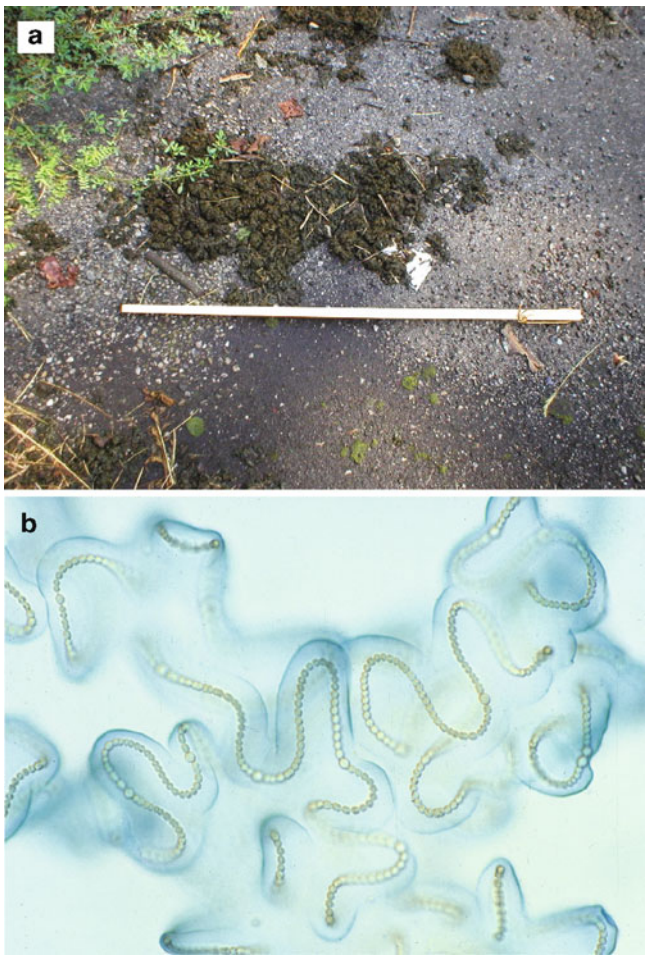

Fig. 18.3 *Nostoc commune* **produces significant amounts of ECM:** (**a**) The organism can form large clusters of a gelatinous biomass when fully hydrated (a *yardstick* is shown for scale); (**b**) ECM2 is readily evident via Alcian Blue staining when *N. commune* is grown in culture. Note the lack of interaction between adjacent ECMs (Field photo courtesy of Jody and Charles Jervis).

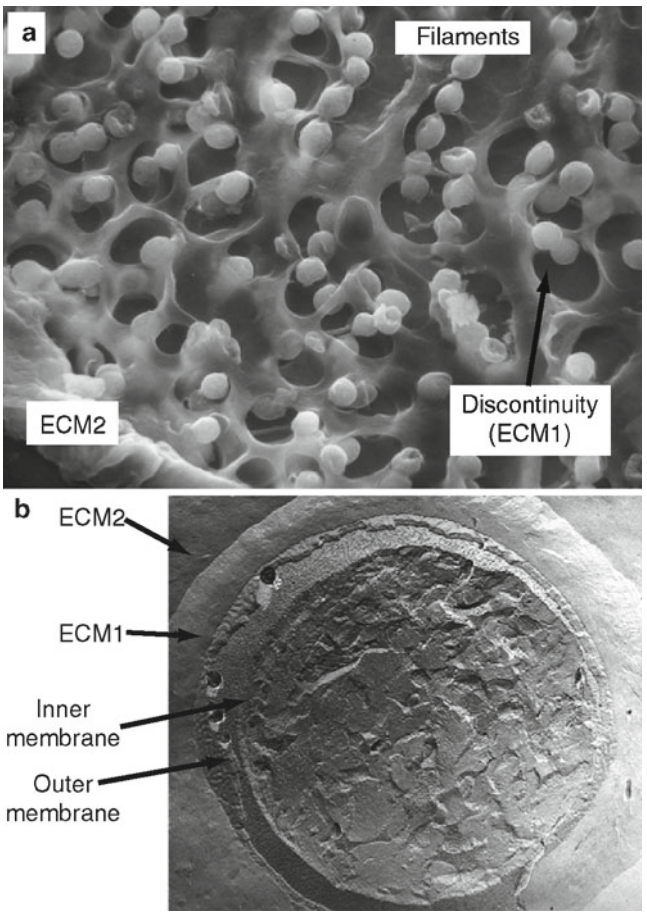

Fig. 18.4 *Nostoc commune* **ECM zones are readily evident using electron microscopy:** (**a**) When submitted to critical point drying and electron microscopy, filaments separate from the ECM2 to create zones of discontinuity, presumably a space between ECM1, which contacts the outer membrane, and ECM2. (**b**) Freeze fracture electron microscopy reveals the orderly composite nature of the cell as well as its extracellular investments. ECM2 is clearly separate from the zone of discontinuity, an ill-defined region termed presently as ECM1. The double membrane system of the cell is also indicated.

The life cycle of *Nostoc commune* was reviewed by Potts (2000) and the properties of its ECMs by Wright et al. (2005); temporal aspects of ECM function are considered here. According to the prevailing environmental conditions, filaments of *Nostoc* may consist of one or more of three cell types: vegetative cells, heterocysts and, in some forms, akinetes. The EPSs of these three cell types may be of different composition (see details later in this chapter) therefore, one must question the differences in the structure and function of their respective ECMs. Through analogy with *Gloeocapsa sanguinea* (above), filaments of *Nostoc commune*, potentially with predominant $ECM1_{VEG}$, and less so $ECM1_{HET}$ and $ECM1_{AKI}$ are embedded within an ECM2 that constitutes the bulk of spherical gelatinous colonies (staining with pH-dependent dyes reveals this bulk is non-homogenous). In comparison to the spherical colonies of *Gloeocapsa* the colonies of *Nostoc* spp. may be much larger (Fig. 18.3). In these colonies, the prospect for filament-filament contact again appears slim, which again raises the questions: is there signaling for such three-dimensional distribution and, if so, why, and how?

Important details of the structure and biochemistry of the ECM of *N. commune* DRH1 and field materials were obtained by Hill et al. (1994a,1997). Freeze fracture electron microscopy provided conclusive evidence for a zone of "discontinuity" at the immediate surface of the cells with chemical and rheological properties distinct from the bulk of the "glycan" (EPS; Fig. 18.4). Interestingly, this zone simply appears as a translucent electron-transparent region when specimens are viewed in the transmitted electron microscope, which was interpreted by others as shrinkage of the EPS from the cells during sample preparation. We hypothesize that the zone of "discontinuity" contains one or more volatile components that are removed during preparation for TEM; low

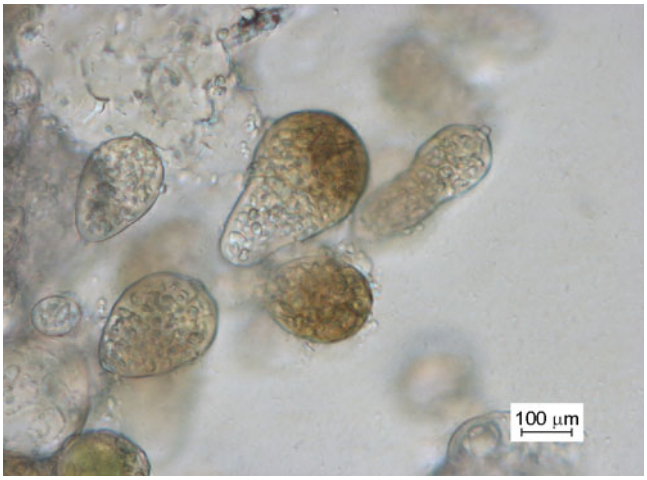

Fig. 18.5 Aseriate growth of *Nostoc commune* results in a different ECM phenotype. Soil-grown isolates from a greenhouse on the campus of Qatar University were cultured in the laboratory. Note the pear-shaped ECM2 encases the entire community. *Amber* colour due to scytonemin.

molecular weight carbohydrates and/or lipid, and water. Note that a similar translucent region was seen in the *Gloeocapsa* confocal images in a fully hydrated state (Fig. 18.2)

At some point in the life cycle of *Nostoc commune* the EPS component(s) of $ECM1_{VEG}$ in a filament (seriate phase) undergoes physical and chemical changes that make it more prominent, rigid and non-expansive. As cell division of the filament continues, the filament is forced to occupy diminishing three-dimensional space due to the increase in its length. Ultimately, this leads to the formation of an ovoid structure, packed with a contorted filament (under pressure?), linked via a single heterocyst (because this cell cannot divide), to another packed ovoid. These "balls" are referred to as the aseriate stage of *N. commune* (Fig. 18.5). Subsequently, for reasons unknown, the $ECM1_{VEG}$ undergoes changes that lead to the bursting of the aseriate masses, and subsequent release and growth of hormogonia and the seriate phase is recovered. In the aseriate phase of *N. commune* in Fig. 18.5 (grown on agar) there is a definite physical polarity of the ECM2 encasing the trichome, with localized production of the photoprotective scytonemin. In the following example, we also discuss polarity and localized production of scytonemin but at the level of a single trichome with ECM1.

Rivularia may also have $ECM1_{VEG}$, and less so $ECM1_{HET}$ and $ECM1_{AKI}$, but here there are some subtle differences. The heterocysts are at one end, and vegetative cells become diminished in size and volume the more distal from the heterocyst so that a tapered filament often ending a hair develops (Chap. 22). The filaments, within a bulk EPS of the ECM2, form semi-spheroid colonies that attach on solid substrates and receive a flow of water and nutrients, typically in streams. In these semi-spheroidal colonies, the heterocystous end of each filament is orientated to the centre of the colony, with the hairs radiating out towards the surface of the colony. With some modifications this is true for other forms in Section IV including marine *Isactis* and *Gardnerula* spp. (Potts 1980). The flux, temporal change in gradients, and dynamics of the ECM in colonies of Rivulariaceae, *Gloeocapsa* cf *sanguinea* and *Nostoc commune*, with respect to stressors, must be very different, and no doubt reflect subtle differences in their respective genomes.

18.1.4 Jelly Bombs

A bizarre example of a cyanobacterial ECM that extends for several square kilometres is found in Storr's Lake; an approximately 2 m deep, orange-coloured, saline, sulphide-rich coastal pond on the island of San Salvador, Bahamas (Paerl et al. 2003). The predominant cyanobacterium is *Aphanothece*, which produces ECM biomass in the order of 10^5 kg km^{-2} (assuming a finite third dimension of 2 m depth). Prevailing wind drives the ECM ashore as a conspicuous carbohydrate-rich foam (Fig. 18.6a). In addition, equally-weird benthic growths of non-heterocystous cyanobacteria form "Jelly Bombs" (or "Ectoplasm Growths" and "Pie Mounds") (Fig. 18.6b, c). These rubbery, gelatinous colonies, when dried and desiccated, easily break a steel scalpel blade upon attempts to obtain sections, emphasizing the pervasive role of water in the structure and function of ECMs.

From the air, Storr's Lake is seen as a bright orange basin against the grey of the island's limestone karst. Flights over many other islands in the Bahamas suggest that counterparts of Storr's Lake are numerous.

18.2 Cyanobacterial Lineage and the ECM

The phylogenetic analysis of prokaryotic evolution utilizing techniques considered to avoid the complexities of horizontal gene transfer (HGT) (Zhaxybayeva et al. 2006) focus on the comparison of so-called "core proteins;" translated products from genes that exhibit reduced levels of HGT (Sanchez-Baracaldo et al. 2005; Shi and Falkowski 2008; Swingley et al. 2008; Gupta 2009; Gupta and Mathews 2010). A study utilizing such techniques placed the Cyanobacteria with the Actinobacteria, Chloroflexi, Firmicutes and *Deinococcus-Thermus* phyla and referred to subsequently as a terrestrial clade (Battistuzzi and Hedges 2009). Lake (2009) analyzed prokaryotic phylogenies based upon the hypothesis that the cyanobacteria (as well as the other double membrane bacteria) were derived from an ancient endosymbiosis event, and suggested that the cyanobacteria were derived from the endosymbiosis of a clostridium and actinobacterium. The characterization

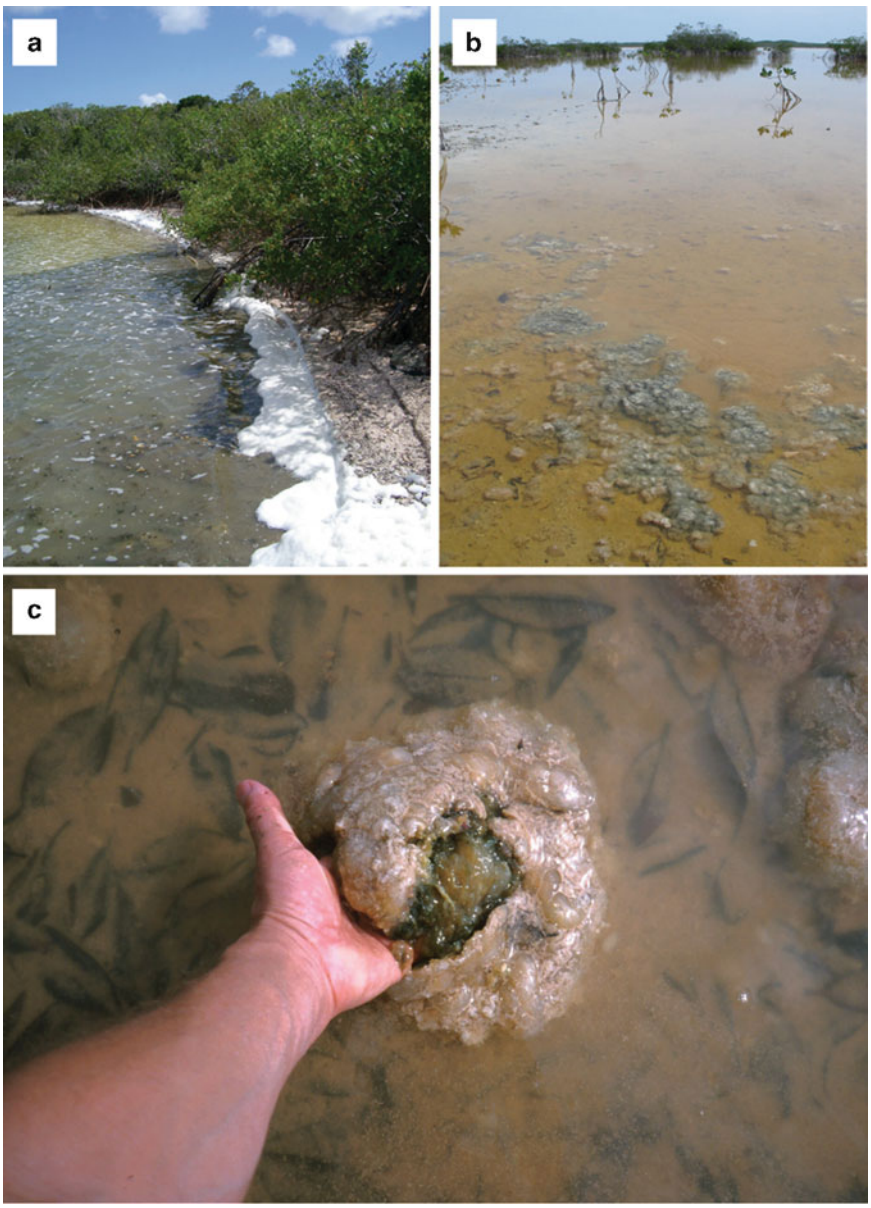

Fig. 18.6 Storr's Lake is a high salt environment with conspicuous levels of ECM materials: (**a**) When washed upon the shore, the EPS forms a foam-like material ("candy floss"); (**b**, **c**) "Jelly bombs" are visible as benthic growths on the water as well as in the sediment (Photos courtesy of H.W. Paerl).

of the protein signatures of 44 sequenced cyanobacteria led to the assignment of three separate clades (Gupta and Mathews 2010). One clade, designated Clade B, contained the majority of known cyanobacteria, including the heterocystous forms. Signature analyses placed the Oscillatoriales in between the orders Nostocales and Chroococcales. Despite the sophistication of such analyses, attempts to mesh data with the realities of structure and function in field populations are relatively rare (Schirrmeister et al. 2011). Emphasis is consistently placed on nucleic acid and protein sequences, with little attention paid to functional evolution. As emphasized elsewhere, the concept of little to zero evolutionary change over geological time (hyperbradytely) is one that is special to the cyanobacteria (Chap. 2) and deserves equal consideration at the levels of metabolism, protein structure/function, and the ECM.

Nonetheless, sequence-based analyses provide insight into the diversity and evolution of the cyanobacterial phylum. This diversity is exemplified by the ubiquitous presence of cyanobacteria in almost all environments that are exposed to a source of light—aquatic, marine and terrestrial. In our work with aerophytic community biofilms (ACBs; also termed subaerial biofilms or SABs), such as those found on solid substrates (stone or roof shingle), the individual communities that develop are both complex and dynamic (Gorbushina and Broughton 2009). Complex by the fact that even cursory phylogenetic analyses with one marker (group I introns), point to a continuum, at least within one morphological subgroup (coccoid cyanobacteria) (D.J. Wright, R.F. Helm, M. Potts, unpublished data). In fact, for a set of *Gloeocapsa* cf *sanguinea* samples evaluated in our laboratory,

there was no obvious indication of the beginning, or end, of the continuum of forms. Such communities are dynamic in the sense that the assemblage of forms inhabiting the biofilm is subject to rapid fluctuations in metabolic activity in response to environmental extremes, imposed in different permutations, over time. For example, desiccation leads to full metabolic arrest, sometimes for protracted periods. During summer, desiccated ACBs reach temperatures of 100°C or greater. At such temperatures, how do cells respond to a transitory rain shower (thunderstorm), accompanied by convective cooling, metabolic activation, and then rapid drying and heating back to 100°C, perhaps multiple times over a period of minutes/hours? Under such environmental extremes, what mechanisms and/or processes are in place to permit a continuum of forms at the genetic level with little apparent evolutionary form change at the geological time scale?

18.3 Components of the Extracellular Matrix

18.3.1 Ecological and Physiological Overview

The most abundant component of a fully hydrated cyanobacterial ECM on a mass basis is water; the second is polysaccharide. Such exopolysaccharides are heterogeneous in carbohydrate composition and may, or may not, have pendant groups such as acetate, sulphate and lactyl groups (Pereira et al. 2009). Generally anionic in nature, these polymers contribute to the structural framework of the ECM, and can be found either loosely surrounding the cells or released to the medium in which the cells are growing. The associated (tightly-bound) polysaccharides are often referred to as sheaths, capsules or slimes, whereas the polysaccharides found in the media are generally referred to as released polysaccharides (RPS). The relationship between the associated and released materials is not fully resolved. Based upon observed differences in chemical characterization, it can be hypothesized that they are derived from separate biosynthetic processes, the result of changes in the monomer and pendant group inputs on the same assembly machinery, or modulation of the outer membrane surface properties.

The roles of the EPS can be summarized using the terrestrial cyanobacterium *Nostoc commune* as an example. Colonies of *N. commune* are a conspicuous feature of many terrestrial soils from the tropics to the polar regions (Potts 2000). Desiccated crusts are brittle and friable, but have the consistency of cartilage when rehydrated. The rapid swelling of desiccated colonies following rainfall is sufficiently striking that it was even the subject of medieval folklore (Potts 1997). This marked capacity for desiccation tolerance (Wright et al. 2005) is linked to the EPS, as this material can inhibit fusion of membrane vesicles during desiccation and freeze drying (Hill et al. 1997), with the anionic polysaccharide providing the repulsive forces that lead to rapid swelling. The glycan is a slowly diffusing/immobilized matrix for a range of secreted enzymes that are active upon rehydration (Peat et al. 1988; Scherer and Potts 1989; Shaw et al. 2003; Morsy et al. 2008), providing a structural and/or molecular scaffold with rheological properties that can accommodate the rapid biophysical and physiological changes that occur upon recovery from stresses such as desiccation. The glycan swells from brittle dried crusts to cartilaginous structures within minutes of rehydration. The matrix contains both lipid- and water-soluble UV radiation-absorbing pigments, protecting the cytosolic components, to some degree, from photodegradation. Finally, although epiphytes colonize the surfaces of *Nostoc* colonies, penetration into the interior is limited due in part to a silicon- and calcium-rich pellicle and inherent resistance of the glycan to enzymatic breakdown. Preliminary structural work on one water-soluble UV-absorbing pigment (released from the glycan by acid hydrolysis) indicated the presence of an oligosaccharide (Bohm et al. 1995), raising the possibility that the pigment may be covalently linked to the glycan in the desiccated state. An understanding of the biochemical and biophysical properties of such biopolymers and the isolation of genes and enzymes required for their synthesis and modification can lead to an understanding of the underlying principles of extremophile stability. Furthermore, one can envision the utilization of such materials for the commercial stabilization of labile agricultural chemicals, food, pharmaceuticals, and/or biomedical materials.

The extracellular matrix is also involved in more large-scale ecological processes. In arid terrestrial environments, cyanobacteria are involved in soil development via the ECM-modulated aggregation of soil particles. These "crusts" help keep soil particles in place and maintain moisture levels for increased lengths of time (Garcia-Pichel and Pringault 2001; Yeager et al. 2007; Chen et al. 2009; Garcia-Pichel and Wojciechowski 2009). Carbon and nitrogen fixation provide long-term soil amendment leading to eventual increases in biological productivity. These subaerial biofilms (SABs) can contribute to weathering of solid surfaces, often leading to detrimental effects as can be seen in the fouling of historical structures (Barberousse et al. 2006; Gorbushina 2007; Macedo et al. 2009). Such SABs are mutualistic in nature as indicated by the shift in *N. punctiforme* growth from filamentous to aseriate when exposed to the yeast *Sarcinomyces petricola* (Gorbushina and Broughton 2009). It is important to emphasize that cyanobacteria, with their associated ECMs, colonize all substrates from plankton (Chap. 20), to solid rock (marine endolithic *Mastigocoleus Kyrtuthrix*, as well as terrestrial chasmoendolithic *Chroococcidiopsis*). In addition they enter into symbiotic associations (Chap. 23). All of these different environments must present unique interfaces for contact between the cyanobacterial ECMs and the environment.

18.3.2 Cyanobacterial Exopolysaccharides

The polysaccharides that provide the bulk of the biomass in the ECM are quite diverse in structure, and the known polysaccharide structures and the factors affecting their production have been summarized (Pereira et al. 2009). While no complete studies have been published that define all variables for a particular strain, a supply of nitrogen and high light generally lead to increased polysaccharide production. Uronic acids are the typical source of the anionic character, along with pyruvate and sulphate moieties, with generally between 4 and 8 different monosaccharides comprising the polysaccharide backbone.

The EPS biosynthetic process in cyanobacteria is ill-defined. The currently available literature on other organisms, as well as data from sequenced genomes, led to the hypothesis that assembly occurs within the inner membrane, with the newly formed products passing through the periplasmic space (Pereira et al. 2009). In the case of filamentous, heterocyst forming cyanobacteria, this must be a highly coordinated event, both biochemically and spatially. Transverse passage through the periplasmic space must occur simultaneously with axial movement of materials between cells (Whitfield and Naismith 2008; Cuthbertson et al. 2009; Flores and Herrero 2010). Once through the periplasmic space, exit through the outer membrane leads to either loosely or tightly bound glycan. The monosaccharides present in cyanobacterial exopolysaccharides are quite diverse and are thought to have temporal and environmental response components (Pereira et al. 2009). While a sigma factor has been identified (Yoshimura et al. 2007) that modulates EPS production in *Anabaena* sp. strain PCC7120 (sheathless and devoid of S-layers), little more is known about the process. Although production rates can be linked to nutrient supplies and cell type (vegetative vs. heterocyst), more specific details are lacking. S-layers (proteins) are foci of calcification (Chap. 16) and could potentially be involved in mineral extraction/utilization processes and also considered a component of the ECM.

Previous reports on the extracellular polysaccharides of cyanobacteria suggest that their structures may not be comparable to those of algae, bacteria or fungi (Morvan et al. 1997; De Philippis and Vincenzini 1998; Helm et al. 2000). The presence/absence of a repeat unit and/or specific polydispersities within isolated polysaccharides are considered unanswered questions. In our work it appeared that the *N. commune* EPS does contain a predominant repeat unit when grown under the specific conditions. There is some degree of flexibility in the sequence of, and control over, the polysaccharide assembly process. As a consequence, cyanobacteria may produce polysaccharides with a specific linkage pattern under one set of conditions but, as the environmental cues change (extreme heat, lack of water, prolonged laboratory culture), the polysaccharide structure may be modified to insure the viability of the organism. This makes structural analysis of the polysaccharides quite challenging, especially for field materials, as they may contain several polysaccharides, each representing the recent environmental history of that location. Such behaviour is not without precedent, as we reported different amounts of nosturonic acid in field-grown materials of *N. commune* from different geographic locations (Helm et al. 2000).

Cyanobacteria such as the Nostocales must have EPS export processes to provide LPS and EPS for at least two different cell types:vegetative cells and heterocysts. As several of these can also have motile (hormogonia), spore-like (akinetes) and aseriate states, additional machinery may be required, or the biosynthetic systems may be modified to permit changes in production rates and formation of different structures/types/forms. Continued research on the biosynthesis of EPS in cyanobacteria will provide new insights into EPS production processes, a better understanding of the role of the periplasmic space in transverse and longitudinal molecular transport processes, the role of EPS in movement (Hoiczyk and Hansel 2000; Garcia-Pichel and Pringault 2001; Read et al. 2007), multicellularity (Lehner et al. 2011) and higher order structures (Garcia-Pichel and Wojciechowski 2009).

18.3.3 eDNA

Biofilm structures in many organisms appear to require the presence of extracellular DNA to form organized matrices. It was reported that eDNA is one of the components of the ECMs of several prokaryotes (Whitchurch et al. 2002), with values on the order of μg eDNA/mL OD_{600} of cells (Wu and Xi 2009). Due to its high phosphorus content, eDNA is important in deep-sea ecosystems (Dell'Anno and Danovaro 2005). Interestingly, extracellular DNA production is not only species dependent, but community dependent as well (Steinberger and Holden 2005). This eDNA is associated generally with initial colonization processes, and DNase treatment strategies can elicit biofilm dispersal. Addition of genomic or salmon sperm DNA to young cultures of *Listeria monocytogenes* could not restore the adherence unless a peptidoglycan was added as well (Harmsen et al. 2010), although this was not the case with *Neisseria meningitides*, where the addition of DNA alone restored biofilm adherence (Lappann et al. 2010). Mature biofilms are significantly more resistant to DNase treament than young colonies, leading to the hypothesis that eDNA is intricately woven into the ECM fabric, where enzymatic breakdown is difficult due to spatial constraints. The most tenable hypothesis on the source of the eDNA is cellular lysis of a subpopulation of cells (Allesen-Holm et al. 2006; Karatan and Watnick 2009). Whether or not eDNA is an integral component of cyanobacterial ECMs and/or what role cell lysis has on this process has yet to be fully investigated.

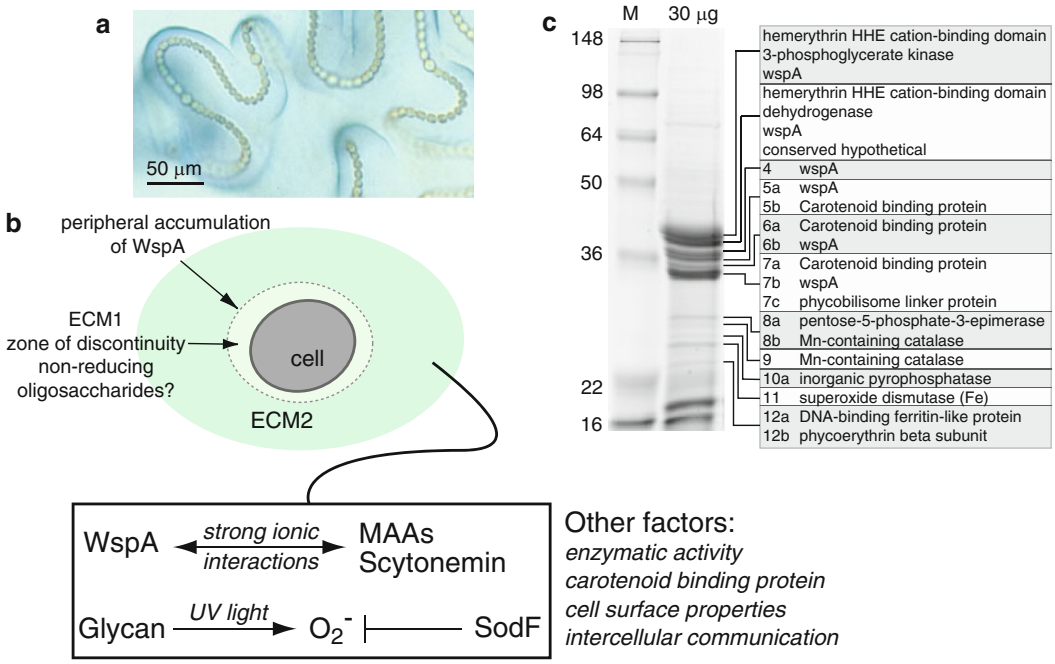

Fig. 18.7 Model of ECM structure and function: (**a**) Alcian blue staining of a liquid culture of *N. commune* DRH1 showing seriate filaments and different rheologies of the glycan (corresponding to different staining levels). (**b**) Model for the extracellular matrix of *N. commune*. WspA is present throughout the glycan, but accumulates at the periphery of a discontinuity in the glycan surrounding cells. The rheology of the ECM2 is determined in part by the glycan and its associations with other matrix components. Glycan releases superoxide radicals that are quenched, in part, by the extracellular superoxide dismutase. (**c**) Isolation of the ECM proteins reveals isoforms of WspA, and several proteins that may have roles in ECM structure and function.

18.3.4 Extracellular Proteins

Proteins released from cellular confinement are often referred to collectively as the secretome. This definition is somewhat problematic for microbial systems that release small peptides, as well as the fact that cell lysis will generate a constellation of proteins that may or may not contribute to cellular responses. Scanlan and Carr (Scanlan and Carr 1988) defined extracellular proteins as those selectively enriched in cell-free media with a mass of greater than 10 kDa and suggested that isolates that are free of light-harvesting biliproteins are true secreted proteins. The filamentous cyanobacterium *Nostoc commune* releases significant quantities of the water-stress protein (WspA) and superoxide dismutase (SodF) as a result of desiccation and UV stresses (Scherer and Potts 1989; Hill et al. 1994a; Wright et al. 2005). Extracts of these materials exhibit xylanase activity. WspA and SodF are both secreted in substantial amounts past the outer membrane of *N. commune* (Shirkey et al. 2000; Ehling-Schulz et al. 2002), yet neither have any recognizable N-terminal signal sequence. A putative C-terminal signal sequence was identified in WspA, but not in SodF (Wright et al. 2005).

The isolated extracellular glycan of *N. commune* DRH1 generates superoxide radicals upon exposure to UV-B irradiation (Shaw et al. 2003), and the superoxide can be scavenged by the superoxide dismutase (SOD) located within the glycan (Shirkey et al. 2000). This observation, as well as the identification of other molecules secreted from *N. commune* led to the hypothesis that the glycan provides the basic lattice of the extracellular matrix within which the central components of WspA and UV-absorbing pigments (mycospsorines, and scytonemin) are distributed (Fig. 18.9). With regard to the different levels of organization, when *N. commune* DRH1 is grown on calcium-supplemented media, the colonies take on a spherical shape and are brown pigmented because of scytonemin. Scytonemin is lipid-soluble and immobilized within the glycan, perhaps even polymerized following secretion from cells. WspA may play a role in modulating the higher order structure of the UV-absorbing pigments in the glycan matrix. It can be further hypothesized that a critical feature of these processes is the specific location of WspA, at the interface of a gel-sol transition boundary close to the cell surface (Fig. 18.7) (Hill et al. 1994a, b, 1997); see Sect. 18.1.3. The extremely hydrophilic nature of the N-terminus of WspA suggests a possible mechanistic basis for its orientation in this transition zone which, on the basis of the volatile nature of the latter during critical point drying (Fig. 18.4), was suggested to be the last repository of liquid, in otherwise desiccated colonies (Hill et al. 1997).

Extracellular phosphatase activities were detected in 35 of 50 different cyanobacterial strains when growth media was assayed at pH 7.6 (Whitton et al. 1991). While it is generally considered that such activities are correlated with maintaining a phosphate supply (Whitton et al. 2005; Mateo et al. 2010), questions pertaining to export, enzymatic control, and half-life remain unanswered. Interestingly, the tyrosine phosphatase IphP of *N. commune* UTEX 584 (Potts et al. 1993) was secreted past the outer membrane of *E. coli* transformants. Thus one can hypothesize that phosphatase is also exported beyond the outer membrane of the host cyanobacterium.

The unicellular freshwater *Synechocystis* PCC 6803 was reported to produce at least seven secreted proteins (Sergeyenko and Los 2000), identified by N-terminal sequencing. Two of these, slr0168 and sll1891 (Nakao et al. 2010), are hypothetical proteins with no annotated conserved domains. Sll0044 is a hypothetical associated with phototaxis (Shin et al. 2008), and Slr0841 is currently listed as a periplasmic protein of unknown function with a weak association with a domain (META) that is associated with motility. Sll1694 is pilin polypeptide PilA1, slr0924 is a periplasmic protein of unknown function (Tic22), associated with the salt stress response (Fulda et al. 2006), and slr1855 is also a hypothetical protein with a strong association with the *N*-acyl-D-glucosamine 2-epimerase (AGE) domain (epimerization during biosynthesis of *N*-acetylneuraminic acid).

A study of freshwater *Oscillatoria* sp. and *Scytonema* sp. found an extracellular phycoerythrin-like protein of approximately 250 kDa that inhibited growth of two green algae, but not other cyanobacteria or eubacteria (Karseno et al. 2009). Data suggest that the extracellular pigment proteins were different from those found intracellularly, supporting the claim that the protein is secreted. This observation calls into question whether or not the presence of pigments in cyanobacterial culture supernatants is due solely to a cell lysis event. Gliding motility and cell-cell contacts in cyanobacteria are also associated with secreted and/or cell surface proteins. The surface of the gliding cyanobacterium *Phormidium uncinatum* contains fibrils on top of its S-layer surface (Smarda et al. 2002) that are comprised of the rod-shaped 66 kDa protein oscillin (Hoiczyk and Baumeister 1997). Evidence supports the role of this protein as a calcium-binding glycoprotein; strains that do not produce the protein lose motility. The marine *Synechococcus* WH8102 requires the S-layer glycoproteins SwmA (130 kDa) and SwmB (1.12 MDa) for non-flagellar movement (Brahamsha 1996; McCarren et al. 2005; McCarren and Brahamsha 2007, 2009), and *Microcystis aeruginosa* PCC 7806 appears to utilize an extracellular glycoprotein, MrpC (15.5 kDa) as well as microvirin (12.2 kDa) for cell-cell contacts (Zilliges et al. 2008). The range of molecular weights in these proteins is rather intriguing. All of these proteins are thought to aggregate into larger quaternary structures, and thus it is possible that the resulting structures are all somewhat similar, only differing in the size of the repeat unit. Although little primary sequence homology exists between these polypeptides, this may be related to "self recognition" processes.

One of the more studied extracellular cyanobacterial proteins is cyanovirin-N (Boyd et al. 1997; Bewley et al. 1998; Botos et al. 2002). This small 11-kDa protein was first isolated from the aqueous cellular extract of laboratory-grown *Nostoc ellipsosporum*. Screening of this polypeptide classified it as an anti-HIV lectin due to its strong binding of the N-linked high-mannose oligosaccharide portion of the gp120 viral coat protein (Yang et al. 1999; Matei et al. 2008; Gronenborn 2009). This protein shares 33% identity with a mannan-binding lectin (MVN) that is involved in cell-cell attachment in *Microcystis aeruginosa* (Kehr et al. 2006). The production of MVN is correlated with microcystin production, but microcystin production is not essential for its expression. Microcystins can control MVN binding partners, which were shown to be both in the sheath and the cell membrane; a scenario that permits cell aggregation.

Several other lectin-type molecules were also isolated from cyanobacteria, including scytovirin, the *Microcystis viridis* lectin (MVL), and *Oscillatoria agardhii* lectin (OAA) (Yamaguchi et al. 1999; Sato et al. 2007). While all of these polypeptides are the subject of studies related to their affinity to carbohydrates for viral therapy, work is expanding slowly with the aim to determine their roles in cyanobacterial physiology. Interestingly, a study on MVL revealed that the homodimer catalyzes the cleavage of chitotriose and chiotetraose to *N*-acetylglucosamine (GlcNAc) (Shahzad-Ul-Hussan et al. 2009). NMR and mutagenesis studies revealed that the mechanism of hydrolysis occurred at a high mannose oligosaccharide binding site, suggesting that the polypeptide can bind one type of oligosaccharide as well as hydrolyze another. It is presumed that further study of cyanobacterial lectins will uncover additional dual roles. Note that the thermostable glycosidases from *N. commune* (Morsy et al. 2008) as well as the xylanohydrolase activities described by Potts (Hill et al. 1994a) may be related to these lectin-like/dual-role polypeptides.

18.3.5 Non-reducing Oligosaccharides

There are several reports that different species of filamentous, heterocyst-forming cyanobacteria produce a series of non-reducing oligosaccharides (Fischer et al. 2006; Pontis et al. 2007; Wieneke et al. 2007). Hot water extraction of whole cells was required to release these substances, calling into question if they are truly extracellular. Unpublished work

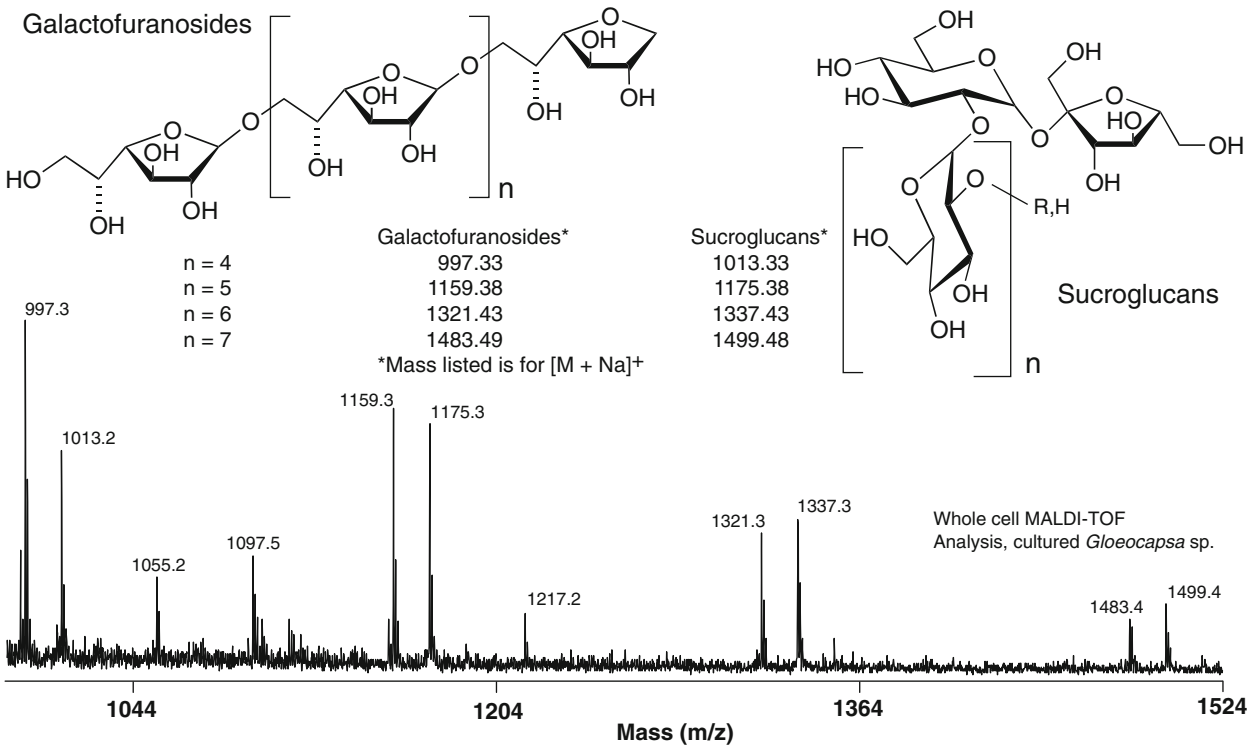

Fig. 18.8 Non-reducing oligosaccharides in cyanobacteria. Structures of the non-reducing galactofuranosides and sucroglucans are shown at the top. MALDI-TOF analysis of roof-isolated and subsequently cultured *Gloeocapsa* indicates that both oligosaccharides are present in this species.

from our laboratory utilizing whole cell MALDI-TOF analysis demonstrates that these compounds are released by the energy associated with the laser pulse (Fig. 18.8) suggesting that these substances may be at the surface of the cells or, at least in a position to be ionized. These structures may be more widespread than previously thought as they are also found in *Gloeocapsa*. In view of the discovery of glycosidase activity for lectins and the ability of lectins to bind specific glycan structures, it can be hypothesized that surface bound lectins adhere avidly to these oligosaccharides promoting a hydrophilic face to the extracellular environment, with extraction from whole cells requiring a hot water extraction. If these "lectins" also possess glycosidase activities (Shahzad-Ul-Hussan et al. 2009), the oligosaccharides could be released from desiccated cells as smaller oligo- or mono-saccharides during the resumption of metabolism. Such complexes may be part of the zone of "discontinuity" observed with desiccation-tolerant cyanobacteria (Fig. 18.3).

18.3.6 EPS-Protein Complexes

The previous discussions of extracellular proteins and the role of the EPS in spatial organization support the distinct possibility that EPS-protein complexes, whether covalent or non-covalent, are also important in ECM physiology. Phytoplankton-secreted polysaccharide-protein complexes of molecular weights greater than 1,000 kDa can act as allelochemicals modulating phytoplankton communities (Yamasaki et al. 2009). Decho and coworkers provided data that further support the role of the EPS in $CaCO_3$ deposition within cyanobacterial mats (Braissant et al. 2009). Such complexes may also be involved in mediating stromatolite formation and stabilization (Havemann and Foster 2008; Foster et al. 2009). Release of these substances was linked to a programmed cell death response in *Trichodesmium* (Berman-Frank et al. 2007).

Association between protein and polysaccharide can occur at the surface adjacent to the outer membrane or in the bulk ECM. Binding at the surface would be related to sheath-type EPS whereas bulk ECM binding would be associated with released polysaccharides. The release of transparent extracellular polysaccharides (TEP) from marine cyanobacteria may be due to production of proteases that not only cause cell death, but release the sheath constituents into the bulk solution as well. The change in cell density leads to settling of the cells, while the EPS materials remain in bulk solution until bound to suspended particle, which leads to deposition. Roles for these substances beyond lithification and carbon recycling are not explored to any great extent for the cyanobacteria.

18.3.7 Low Molecular Weight Substances (Secondary Metabolites)

The total number of "secondary metabolites" produced by cyanobacteria is in the thousands, with many being present in the ECM; either released by living cells or from cell lysis of a subpopulation. Compounds range in size from the neurotoxin β-methylamino-L-alanine (BMAA) to cyclic peptides such as microviridin (Van Wagoner et al. 2007). Their roles in situ are not well understood, with hypotheses including defense strategies, gene regulation and cell-cell communication (Schatz et al. 2007).

Why so many small molecules? Such numbers are suggestive of a combinatorial library where each substance provides benefit to the organism for a particular environmental stress. Are there so many secondary metabolites because there are so many possible environmental perturbations? Is the library essentially a history of the organism with contributions from both mutational and gene transfer processes? This issue was the focus for the plant biology community for some time (Firn and Jones 2000, 2009; Fischbach and Clardy 2007) and is worth pondering from cyanobacterial and ecological perspectives.

When present as a member of a community, one can argue that four choices are possible: interaction, competition, neutrality or evasion. Interactions are positive as seen in cyanobacterial mats and stromatolites, and negative as exemplified by cyanobacterial phages or the toxicity of many cyanobacterial products towards humans. Neutrality is not truly an option as the footprint of any organism is presumably detected by another, especially when resources become scarce. Evasion requires movement and/or adoption of a state that does not permit detection. Based upon evidence presented to date, the best argument for cyanobacterial toxins is that they initially served a non-toxic, alternative purpose well before they impacted large-scale ecological niches (Leao et al. 2009, 2010) or considered contaminants in the human water supply (geosmin and 2-methylisoborneol) (Izaguirre et al. 1982; Agger et al. 2008).

As the cyanobacteria clearly predate eukaryotes, theories that support "secondary" metabolite production as a means to control grazing appear untenable in comparison to siderophoric activities and/or cell signaling activities (Rantala et al. 2004). Questions that arise are whether or not there are modifications to the molecule, if so are these related to random mutations within specific gene sequences, and whether or not the resulting product provides an advantage during subsequent stresses. This hypothesis would then require that variability of metabolite production in closely related strains would be the result of HGT, localized evolutionary pressures (resulting in gene loss), and potentially community-related signaling processes.

The most tenable hypothesis concerning the role of small extracellular molecules in cyanobacteria is that their initial advantage was for signaling purposes, such as PatS (Yoon and Golden 1998). Studies on microcystins in cyanobacterial populations in Antarctica does not provide much support for prevention of grazing and/or elimination of competing phytoplankton (Wood et al. 2008), as competition at these sites is minimal. Modulations of protease and phosphatase activities, whether intracellular or intracellular, are much stronger arguments. Such strategies may provide for adjustment to environmental stresses such as UV-light, changes in temperature, moisture, salinity, and the presence of phages.

Phages can be induced into a lytic phase, which results in lysis of the host cell, or a lysogenic phase, where the viral genome is maintained in the prophage state as the host grows and multiplies (Long et al. 2008). The prevailing thought with regards to the phage lifestyle is that that they can be both pathogenic (lytic) and mutualistic (lysogenic/prophage). Phages can deliver genes via horizontal gene transfer, which is a potential source of genes required to exit exposure to precarious environmental conditions that could compromise the viability of the organism or community (Lindell et al. 2004; Sullivan et al. 2006, 2009). Lysogeny has been correlated with conditions of low microbe abundance, and is considered an adaptive response to survive low host growth rates. A study with *E. coli*, *P. aeruginosa* and soil isolates containing cyanobacteria found a correlation between acyl-homoserine lactones (autoinduction/quorum-sensing molecules) and phage production (Ghosh et al. 2009). Thus there may be a link between cyanobacterial-derived extracellular signalling molecules and phage physiology.

18.4 Autoinduction Systems in Cyanobacteria

Autoinduction is a process by which a compound released to the extracellular space modulates cellular behaviour (Nealson et al. 1970; Eberhard et al. 1981; Fuqua et al. 1994). Subsequently defined as quorum sensing (Fuqua et al. 1994), this messenger system is found in most Gram-negative bacteria (Whitehead et al. 2001; Waters and Bassler 2005; Dickschat 2010). Typically these molecules are detected when bacteria are grown to high cell density in the laboratory. An example of a field condition with high cell density would be a microbial mat or a stromatolite, or dense planktonic bloom; situations where cyanobacteria are present. While there are several types of quorum sensing molecules, acyl-homoserine lactones are the most commonly studied, and can accumulate to mM concentrations in the extracellular space (Nadell et al. 2009).

Control of quorum sensing compound synthesis is of intense interest in the microbial community. In the strictest definition, current dogma posits that when the intracellular concentration of a QS molecule reaches a specific level, a change in gene expression occurs that leads to a change in

community behaviour. Over 70 different species of bacteria are known to produce AHLs (Waters and Bassler 2005), and recent phylogenetic analyses suggest that the QS signaling pathway is present in 68 different bacterial genomes (Case et al. 2008), but not in the Archaea.

Cyanobacteria are observed to behave in a cooperative manner in community development, and hence it is logical to assume, that like most Gram-negative bacteria, they utilize sensing / autoinduction processes as well. The original definition of quorum sensing is that bacterial cells assess their environment by the production of autoinducer molecules that can modulate gene expression. This results in a group of cells acting as a behavioural unit (Hense et al. 2007). Known autoinducers include small peptides, a ribose derivative, or acyl homoserine lactones (AHLs). While QS has morphed over time to be considered a high cell density behaviour, QS processes are essentially sentinel systems invoked by single cells to query the local environment (Redfield 2002). Diffusion of the molecules away from the cell does not elicit a response. However, if the molecules remain in close proximity to the cell or a cluster of cells, their presence elicits a change in gene expression leading the production of additional secretory molecules such as degradative enzymes, siderophores, antibiotics or surfactants. This concept was extended recently to account for complex communities and the spatial distribution of cells within these communities and is termed efficiency sensing (Hense et al. 2007).

In the efficiency-sensing hypothesis, autoinducers are probes used to assess continually cell density, the spatial distribution of cells, and the mass transfer properties of the local environment. If autoinducer concentrations reach a specific threshold level, conditions are appropriate for the production of costlier extracellular effector molecules. By definition, this is not a cooperative or coordinated process; cells are acting individually but can act in coordinated fashion when neighboring cells have the same autoinducer system (Hense et al. 2007). In organisms that produce significant quantities of extracellular polysaccharides, clonal colonies can develop permitting group behaviour through positive feedback mechanisms. Such processes protect the developing colony through paracrine signaling while also promoting clonal diversity.

Boedicker et al. (2009) found that individual cells of *P. aeruginosa* confined to femtolitres of media can activate the quorum sensing pathway. Using transformed *E. coli* in a model biofilm (Timp et al. 2009), it was concluded that cell-to-cell signaling is diffusion-controlled with a spatial distribution of autoinducers. Gantner et al. evaluated rhizobacteria on tomato and wheat roots and found that communication can occur in small groupings of cells and over ranges of up 78 μm (Gantner et al. 2006). Such results suggest that autocrine and paracrine signalling occurring in bacteria is a consequence of individual cells, resulting in community-like responses.

Some cyanobacterial specialists have assumed that the traditional quorum sensing-type processes are not present (Haselkorn 2008). Hence the report of an acyl-homoserine lactone in *Gloeothece* PCC6909 was somewhat of a surprise (Sharif et al. 2008). This discovery supports the concept that some cyanobacteria can assess and respond to AHLs in their environment. (Romero et al. 2008, 2011). The recent demonstration of a *p*-coumaroyl homoserine lactone in photosynthetic bacteria (*Rhodopseudomonas palustris, Bradyrhizobium* sp. BTAi1) (Schaefer et al. 2008), the presence of extracellular "life cycle governing factors" (LCGFs) in *N. punctiforme* (Liaimer et al. 2011) as well as the results of others (Decho et al. 2009) suggest that the presence of sentinel sensing molecules in cyanobacteria deserves critical evaluation. Molecules such as geosmin and 2-methylisoborneol may indeed be part of the environmental sensing network in cyanobacteria.

18.5 Oxidative Stress and Community Diversity

The diversity of cyanobacterial communities suggests that mechanisms are in place to generate this diversity, with the overall goal of surviving in a natural environment. The "insurance hypothesis" for microbial systems posits that a RecA-mediated process leads to diversification, and these processes are enhanced in clonal biofilms (Boles et al. 2004, 2005). Such processes can lead to mutualistic behaviour where one variant can aid in the survival of another, eventually providing long-term stability of the community (Hillesland and Stahl 2010). The fact that clonal bacterial biofilms can generate measurable diversity within hours suggests a robust and rapidly mobilized mechanism. A more recent study of the insurance hypothesis led to the discovery that the mechanism is grounded in oxidative stress (Boles and Singh 2008). Endogenous oxidative stress leads to double strand DNA breaks, which when repaired, with some degree of inaccuracy, provides diversity. Subsequent environmental stresses result in the demise of some mutants and the advancement of others (Fig. 18.9).

The ECM of *Nostoc commune* contains a substantial quantity of superoxide dismutase (SOD), an enzyme that can be stored within the desiccated ECM for at least a decade; becoming active upon rehydration (Fig. 18.7) (Shirkey et al. 2000). The dismutation reaction forms both oxygen and hydrogen peroxide from the superoxide anion radical. While this dismutation reaction can occur non-enzymatically, SOD enzymes typically exhibit high turnover numbers, thereby keeping free radical concentrations low. When exposed to UV-light, the EPS of *N. commune* is a major source of free radicals (Shaw et al. 2003). Thus, while the EPS is protective under some conditions, it can be damaging under others,

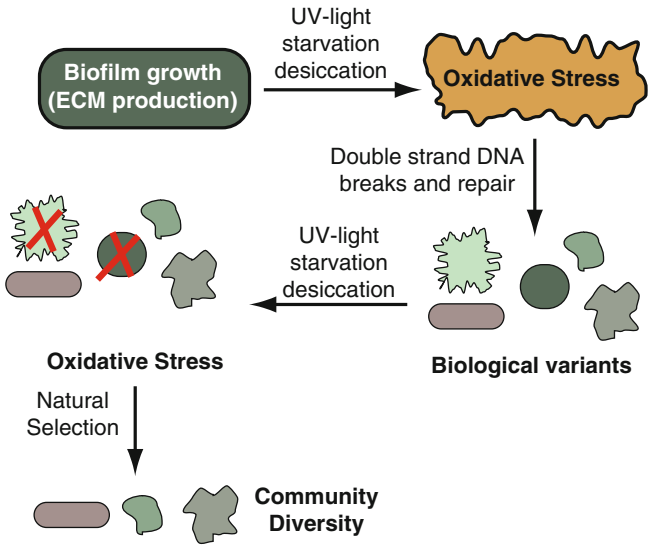

Fig. 18.9 The stochastic nature of microbial biodiversity. The Gram-positive *Bacillus subtilis* can undergo phenotypic transformations that can lead to the cell types shown. Note that cyanobacteria have several analogous cell types including akinetes, hormogonia, vegetative cells (EPS producer) and heterocysts (EPS, death or surfactin producer?). Each of these cell types, upon exposure to oxidative stress, may respond differently, especially if their abilities to modulate oxidant levels differ. Thus the generation of biological variants can be likened to a "wheel of fortune" where each environmental perturbation can lead to a different variant (Concept adapted from Lopez and Kolter 2010).

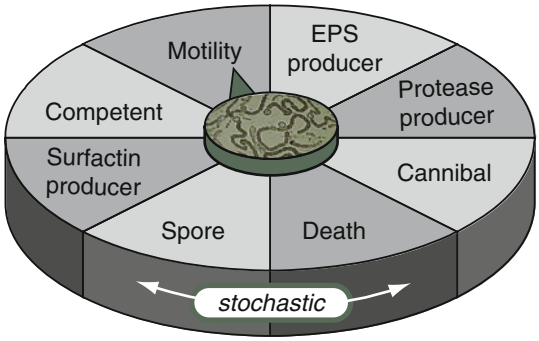

Fig. 18.10 Community diversity through oxidative stress. Biofilms are exposed to an environmental shift that causes oxidative stress. The ECM is both a source (bulk glycan) and dampener (UV photoprotective pigments and SOD) of oxidant levels. Oxidative stress leads to double strand DNA breaks and repair processes (RecA-mediated) lead to biological variants. These variants are exposed to additional stresses that select for the most robust. The net result is a more diverse community (Adapted from Boles and Singh 2008).

with SOD providing free radical buffering capacity. While hydrogen peroxide can be quenched by catalases (note presence in the extracellular proteome of *N. commune*, Fig. 18.7), it could also be the molecule acting on DNA to provide oxidative stress, initiating double strand break repair processes and subsequent genome diversity.

Cell fate in a biofilm community will be dependent upon the location of a cell within the biofilm and the signals it receives from the environment. While much emphasis has been placed in intracellular signaling processes in cyanobacteria, evidence is clearly mounting that there is an extracellular component as well. The Gram-positive *Bacillus subtilis* has been shown to undergo phenotypic transformations that can lead to the cell types shown in Fig. 18.9 (Lopez et al. 2009a, b; Lopez and Kolter 2010). Note that cyanobacteria have several analogous cell types including akinetes (spore), hormogonia (motility), vegetative cells (EPS producer), necridia, and heterocysts (EPS, death or surfactin producer?). Each of these cell types, upon exposure to oxidative stress may respond differently, especially if their abilities to modulate oxidant levels are different. Thus the generation of biological variants can be likened to a "wheel of fate", where each environmental perturbation can lead to a different variant. Survival is thus dependent upon the ability of the variant to survive the next environmental stress (Fig. 18.10).

18.6 Future Prospects

The extracellular matrices of cyanobacteria are both complex and dynamic. Anionic polysaccharides provide a framework for the regio-deposition of an array of proteins, small molecules and potentially nucleic acids resulting in a four-dimensional matrix that permits survival under a particular set of environmental conditions. Adjustments to the ECM are continual and are hypothesized as providing the reagents necessary to yield genetic diversity.

Cyanobacteria can be found in all environments that receive light. Increases in the Earth's surface temperature are expected to favour cyanobacteria (Paerl and Huisman 2008) in aquatic ecosystems, potentially having dramatic effects on the safety of drinking water. Changes in rainfall and core land temperatures will also modulate cyanobacterial populations, although the net effect of increased temperatures and rising sea levels will probably result in more spatially-dependent changes in microbial populations/ecotypes (Green et al. 2008; Koeppel et al. 2008; Ward et al. 2008). Will terrestrial-, freshwater-, or marine-based changes in climate and carbon dioxide levels result in changes in cyanobacterial communities? Efforts to understand cyanobacterial physiology are important for understanding the interactive roles cyanobacteria play in localized as well as global processes.

Over 75 cyanobacterial genomic and metagenomic projects are completed or are currently underway. This number reflects the biological and ecological importance of these microbes. However, considering the efforts underway at the genomic level, it is extremely surprising to see that cyanobacterial "metabolomic" projects initially appeared in the literature only recently (Eisenhut et al. 2008; Wase and Wright 2008; Krall et al. 2009; Bennette et al. 2011). The cyanobacterial

research community would benefit greatly from datasets characterizing extracellular metabolite pools and how they change upon environmental stress and/or genetic perturbation (Baran et al. 2010, 2011). Even if we confine our discussion of cyanobacteria to just those species presently available from culture collections, we have an exceedingly poor understanding of the breadth and diversity of molecules within these collections and how they change as the result of culture conditions. Such baseline evaluation is critical for assessing chemotypic changes associated with environmental and/or genetic inputs. Profiling efforts that initially provide broad screens will provide the general overview required for quantitative studies that target specific metabolites, processes, and/or cell types. Such datasets are crucial to rationally directing molecular biology toward new and important genes and enzymatic processes (Balskus and Walsh 2009, 2010; Jones et al. 2009, 2010; Leikoski et al. 2009, 2010; Sivonen et al. 2010; Spence et al. 2012).

While studies in the evolutionary pressures and processes continue to provide insights into cyanobacterial history and diversity, such efforts can only suggest a physiological understanding of why a particular ecotype is advantageous over another. Metagenomic screens cannot provide direct insight into survival mechanisms or community behaviour either. Evaluation of stress responses in microbial communities in real time requires interrogating the metagenomic outputs: proteins, secondary metabolites and polysaccharides. These molecules have a much higher "resolving power" than nucleic acids; hence the emphasis on biomarkers in biomedical research. Analysis of the molecular species (glycans, small molecules, and proteins) placed outside the cyanobacterial cell will provide the information necessary to test the insurance hypothesis and if indeed oxidative stress is the source of genetic diversity. Evaluation of cyanobacterial growth at the level of extracellular molecular species will provide new insights into the species/ecotype concept, lead us to a better understanding of the core cyanobacterial genes, offer fundamental units (molecules) of cyanobacterial diversity and shed additional light on how/why biosynthetic pathways diverge.

References

Agger SA, Lopez-Gallego F, Hoye TR, Schmidt-Dannert C (2008) Identification of sesquiterpene synthases from *Nostoc punctiforme* PCC 73102 and *Nostoc* sp. strain PCC 7120. J Bacteriol 190: 6084–6096

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2008) Molecular biology of the cell, 4th edn. Garland Science, New York, 1359 pp

Allesen-Holm M, Barken KB, Yang L, Klausen M, Webb JS, Kjelleberg S et al (2006) A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. Mol Microbiol 59:1114–1128

Balskus EP, Walsh CT (2009) An enzymatic cyclopentyl[b]indole formation involved in scytonemin biosynthesis. J Am Chem Soc 131:14648–14649

Balskus EP, Walsh CT (2010) The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. Science 329:1653–1656

Baran R, Bowen BP, Bouskill NJ, Brodie EL, Yannone SM, Northen TR (2010) Metabolite identification in *Synechococcus* sp. PCC 7002 using untargeted stable isotope assisted metabolite profiling. Anal Chem 82:9034–9042

Baran R, Bowen BP, Northen TR (2011) Untargeted metabolic footprinting reveals a surprising breadth of metabolite uptake and release by *Synechococcus* sp. PCC 7002. Mol Biosyst 7:3200–3206

Barberousse H, Ruiz G, Gloaguen V, Lombardo RJ, Djediat C, Mascarell G et al (2006) Capsular polysaccharides secreted by building facade colonisers: characterisation and adsorption to surfaces. Biofouling 22:361–370

Battistuzzi FU, Hedges SB (2009) A major clade of prokaryotes with ancient adaptations to life on land. Mol Biol Evol 26:335–343

Bennette NB, Eng JF, Dismukes GC (2011) An LC-MS-based chemical and analytical method for targeted metabolite quantification in the model cyanobacterium *Synechococcus* sp. PCC 7002. Anal Chem 83:3808–3816

Berman-Frank I, Rosenberg G, Levitan O, Haramaty L, Mari X (2007) Coupling between autocatalytic cell death and transparent exopolymeric particle production in the marine cyanobacterium *Trichodesmium*. Environ Microbiol 9:1415–1422

Bewley CA, Gustafson KR, Boyd MR, Covell DG, Bax A, Clore GM et al (1998) Solution structure of cyanovirin-N, a potent HIV-inactivating protein. Nat Struct Biol 5:571–578

Boedicker JQ, Vincent ME, Ismagilov RF (2009) Microfluidic confinement of single cells of bacteria in small volumes initiates high-density behavior of quorum sensing and growth and reveals its variability. Angew Chem Int Ed Engl 48:5908–5911

Bohm GA, Pfleiderer W, Boger P, Scherer S (1995) Structure of a novel oligosaccharide-mycosporine amino acid ultraviolet A/B sunscreen from the terrestrial cyanobacterium *Nostoc commune*. J Biol Chem 270:8536–8539

Boles BR, Singh PK (2008) Endogenous oxidative stress produces diversity and adaptability in biofilm communities. Proc Natl Acad Sci USA 105:12503–12508

Boles BR, Thoendel M, Singh PK (2004) Self-generated diversity produces "insurance effects" in biofilm communities. Proc Natl Acad Sci USA 101:16630–16635

Boles BR, Thoendel M, Singh PK (2005) Genetic variation in biofilms and the insurance effects of diversity. Microbiology 151:2816–2818

Botos I, O'Keefe BR, Shenoy SR, Cartner LK, Ratner DM, Seeberger PH et al (2002) Structures of the complexes of a potent anti-HIV protein cyanovirin-N and high mannose oligosaccharides. J Biol Chem 277:34336–34342

Boyd MR, Gustafson KR, McMahon JB, Shoemaker RH, Okeefe BR, Mori T et al (1997) Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrob Agents Chemother 41:1521–1530

Brahamsha B (1996) An abundant cell-surface polypeptide is required for swimming by the nonflagellated marine cyanobacterium *Synechococcus*. Proc Natl Acad Sci USA 93:6504–6509

Braissant O, Decho AW, Przekop KM, Gallagher KL, Glunk C, Dupraz C et al (2009) Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat. FEMS Microbiol Ecol 67:293–307

Case RJ, Labbate M, Kjelleberg S (2008) AHL-driven quorum-sensing circuits: their frequency and function among the Proteobacteria. ISME J 2:345–349

Cates ME, Marenduzzo D, Pagonabarraga I, Tailleur J (2010) Arrested phase separation in reproducing bacteria creates a generic route to pattern formation. Proc Natl Acad Sci USA 107:11715–11720

Chen LZ, Wang GH, Hong S, Liu A, Li C, Liu YD (2009) UV-B-induced oxidative damage and protective role of exopolysaccharides in desert cyanobacterium *Microcoleus vaginatus*. J Integr Plant Biol 51:194–200

Costerton WJ (2004) A short history of the development of the biofilm concept. In: Ghannoum M, O'Toole GA (eds) Microbial biofilms. ASM Press, Washington, DC, pp 4–19

Cuthbertson L, Mainprize IL, Naismith JH, Whitfield C (2009) Pivotal roles of the outer membrane polysaccharide export and polysaccharide copolymerase protein families in export of extracellular polysaccharides in gram-negative bacteria. Microbiol Mol Biol Rev 73:155–177

De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22:151–175

Decho AW, Visscher PT, Ferry J, Kawaguchi T, He L, Przekop KM et al (2009) Autoinducers extracted from microbial mats reveal a surprising diversity of N-acylhomoserine lactones (AHLs) and abundance changes that may relate to diel pH. Environ Microbiol 11:409–420

Dell'Anno A, Danovaro R (2005) Extracellular DNA plays a key role in deep-sea ecosystem functioning. Science 309:2179–2179

Dickschat JS (2010) Quorum sensing and bacterial biofilms. Nat Prod Rep 27:343–369

Dittrich M, Sibler S (2005) Cell surface groups of two picocyanobacteria strains studied by zeta potential investigations, potentiometric titration, and infrared spectroscopy. J Colloid Interface Sci 286: 487–495

Eberhard A, Burlingame AL, Eberhard C, Kenyon GL, Nealson KH, Oppenheimer NJ (1981) Structural identification of autoinducer of *Photobacterium fischeri* luciferase. Biochemistry 20:2444–2449

Ehling-Schulz M, Schulz S, Wait R, Gorg A, Scherer S (2002) The UV-B stimulon of the terrestrial cyanobacterium *Nostoc commune* comprises early shock proteins and late acclimation proteins. Mol Microbiol 46:827–843

Eisenhut M, Huege J, Schwarz D, Bauwe H, Kopka J, Hagemann M (2008) Metabolome phenotyping of inorganic carbon limitation in cells of the wild type and photorespiratory mutants of the cyanobacterium *Synechocystis* sp. strain PCC 6803. Plant Physiol 148:2109–2120

Firn RD, Jones CG (2000) The evolution of secondary metabolism – a unifying model. Mol Microbiol 37:989–994

Firn RD, Jones CG (2009) A Darwinian view of metabolism: molecular properties determine fitness. J Exp Bot 60:719–726

Fischbach MA, Clardy J (2007) One pathway, many products. Nat Chem Biol 3:353–355

Fischer D, Geyer A, Loos E (2006) Occurrence of glucosylsucrose alpha-D-glucopyranosyl-(1->2)-alpha-D-glucopyranosyl-(1->2)-beta-D-fructofuranoside and glucosylated homologues in cyanobacteria – Structural properties, cellular contents and possible function as thermoprotectants. FEBS J 273:137–149

Flores E, Herrero A (2010) Compartmentalized function through cell differentiation in filamentous cyanobacteria. Nat Rev Microbiol 8:39–50

Foster JS, Green SJ, Ahrendt SR, Golubic S, Reid RP, Hetherington KL et al (2009) Molecular and morphological characterization of cyanobacterial diversity in the stromatolites of Highborne Cay, Bahamas. ISME J 3:573–587

Fulda S, Mikkat S, Huang F, Huckauf J, Marin K, Norling B et al (2006) Proteome analysis of salt stress response in the cyanobacterium *Synechocystis* sp strain PCC 6803. Proteomics 6:2733–2745

Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176:269–275

Gantner S, Schmid M, Durr C, Schuhegger R, Steidle A, Hutzler P et al (2006) In situ quantitation of the spatial scale of calling distances and population density-independent N-acylhomoserine lactone-mediated communication by rhizobacteria colonized on plant roots. FEMS Microbiol Ecol 56:188–194

Garcia-Pichel F, Pringault O (2001) Microbiology – cyanobacteria track water in desert soils. Nature 413:380–381

Garcia-Pichel F, Wojciechowski MF (2009) The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PLoS One 4:e7801

Geitler L (1932) Cyanophyceae. Rabenhorst's Krytogamen-Flora von Deutschland, Österreich und der Schweiz 14. Akademische Verlagsgesellschaft, Leipzig, 1196 pp

Ghosh D, Roy K, Williamson KE, Srinivasiah S, Wommack KE, Radosevich M (2009) Acyl-homoserine lactones can induce virus production in lysogenic bacteria: an alternative paradigm for prophage induction. Appl Environ Microbiol 75:7142–7152

Gorbushina AA (2007) Life on the rocks. Environ Microbiol 9:1613–1631

Gorbushina AA, Broughton WJ (2009) Microbiology of the atmosphere-rock interface: how biological interactions and physical stresses modulate a sophisticated microbial ecosystem. Annu Rev Microbiol 63:431–450

Green JL, Bohannan BJ, Whitaker RJ (2008) Microbial biogeography: from taxonomy to traits. Science 320:1039–1043

Gronenborn AM (2009) Protein acrobatics in pairs – dimerization via domain swapping. Curr Opin Struct Biol 19:39–49

Gupta RS (2009) Protein signatures (molecular synapomorphies) that are distinctive characteristics of the major cyanobacterial clades. Int J Syst Evol Microbiol 59:2510–2526

Gupta RS, Mathews DW (2010) Signature proteins for the major clades of cyanobacteria. BMC Evol Biol 10:24

Harmsen M, Lappann M, Knochel S, Molin S (2010) Role of extracellular DNA during biofilm formation by *Listeria monocytogenes*. Appl Environ Microbiol 76:2271–2279

Haselkorn R (2008) Cell-cell communication in filamentous cyanobacteria. Mol Microbiol 70:783–785

Havemann SA, Foster JS (2008) Comparative characterization of the microbial diversities of an artificial microbialite model and a natural stromatolite. Appl Environ Microbiol 74:7410–7421

Helm RF, Huang Z, Edwards D, Leeson H, Peery W, Potts M (2000) Structural characterization of the released polysaccharide of desiccation-tolerant *Nostoc commune* DRH-1. J Bacteriol 182:974–982

Hense BA, Kuttler C, Muller J, Rothballer M, Hartmann A, Kreft J-U (2007) Does efficiency sensing unify diffusion and quorum sensing? Nat Rev Microbiol 5:230–239

Hill DR, Hladun SL, Scherer S, Potts M (1994a) Water-stress proteins of *Nostoc commune* (Cyanobacteria) are secreted with UV-A/B-absorbing pigments and associate with 1,4-beta-D-xylanohydrolase activity. J Biol Chem 269:7726–7734

Hill DR, Peat A, Potts M (1994b) Biochemistry and structure of the glycan secreted by desiccation-tolerant *Nostoc commune* (Cyanobacteria). Protoplasma 182:126–148

Hill DR, Keenan TW, Helm RF, Potts M, Crowe LM, Crowe JH (1997) Extracellular polysaccharide of *Nostoc commune* (cyanobacteria) inhibits fusion of membrane vesicles during desiccation. J Appl Phycol 9:237–248

Hillesland KL, Stahl DA (2010) Rapid evolution of stability and productivity at the origin of a microbial mutualism. Proc Natl Acad Sci USA 107:2124–2129

Hoiczyk E, Baumeister W (1997) Oscillin, an extracellular, Ca2+-binding glycoprotein essential for the gliding motility of cyanobacteria. Mol Microbiol 26:699–708

Hoiczyk E, Hansel A (2000) Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J Bacteriol 182:1191–1199

Izaguirre G, Hwang CJ, Krasner SW, McGuire MJ (1982) Geosmin and 2-methylisoborneol from cyanobacteria in three water supply systems. Appl Environ Microbiol 43:708–714

Jones AC, Gu L, Sorrels CM, Sherman DH, Gerwick WH (2009) New tricks from ancient algae: natural products biosynthesis in marine cyanobacteria. Curr Opin Chem Biol 13:216–223

Jones AC, Monroe EA, Eisman EB, Gerwick L, Sherman DH, Gerwick WH (2010) The unique mechanistic transformations involved in the biosynthesis of modular natural products from marine cyanobacteria. Nat Prod Rep 27:1048–1065

Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73:310–347

Karseno HK, Bamba T, Dwi S, Mahakhant A, Yoshikawa T et al (2009) Extracellular phycoerythrin-like protein released by freshwater cyanobacteria *Oscillatoria* and *Scytonema* sp. Biotechnol Lett 31:999–1003

Kehr JC, Zilliges Y, Springer A, Disney MD, Ratner DD, Bouchier C et al (2006) A mannan binding lectin is involved in cell-cell attachment in a toxic strain of *Microcystis aeruginosa*. Mol Microbiol 59:893–906

Koeppel A, Perry EB, Sikorski J, Krizanc D, Warner A, Ward DM et al (2008) Identifying the fundamental units of bacterial diversity: a paradigm shift to incorporate ecology into bacterial systematics. Proc Natl Acad Sci USA 105:2504–2509

Krall L, Huege J, Catchpole G, Steinhauser D, Willmitzer L (2009) Assessment of sampling strategies for gas chromatography-mass spectrometry (GC-MS) based metabolomics of cyanobacteria. J Chromatogr B Anal Technol Biomed Life Sci 877:2952–2960

Kumar KV, Kumaran A (2005) Voronoi cell volume distribution and configuration entropy of hard-spheres. J Chem Phys 123:114501

Lake JA (2009) Evidence for an early prokaryotic endosymbiosis. Nature 460:967–971

Lappann M, Claus H, van Alen T, Harmsen M, Elias J, Molin S et al (2010) A dual role of extracellular DNA during biofilm formation of *Neisseria meningitidis*. Mol Microbiol 75:1355–1371

Leao PN, Vasconcelos MT, Vasconcelos VM (2009) Allelopathy in freshwater cyanobacteria. Crit Rev Microbiol 35:271–282

Leao PN, Pereira AR, Liu WT, Ng J, Pevzner PA, Dorrestein PC, Konig GM, Vasconcelos VM, Gerwick WH (2010) Synergistic allelochemicals from a freshwater cyanobacterium. Proc Natl Acad Sci USA 107:11183–11188

Lehner J, Zhang Y, Berendt S, Rasse TM, Forchhammer K, Maldener I (2011) The morphogene AmiC2 is pivotal for multicellular development in the cyanobacterium *Nostoc punctiforme*. Mol Microbiol 79:1655–1669

Leikoski N, Fewer DP, Sivonen K (2009) Widespread occurrence and lateral transfer of the cyanobactin biosynthesis gene cluster in cyanobacteria. Appl Environ Microbiol 75:853–857

Leikoski N, Fewer DP, Jokela J, Wahlsten M, Rouhiainen L, Sivonen K (2010) Highly diverse cyanobactins in strains of the genus *Anabaena*. Appl Environ Microbiol 76:701–709

Liaimer A, Jenke-Kodama H, Ishida K, Hinrichs K, Stangeland J, Hertweck C, Dittmann E (2011) A polyketide interferes with cellular differentiation in the symbiotic cyanobacterium *Nostoc punctiforme*. Environ Microbiol Rep 3: 550–558

Lindell D, Sullivan MB, Johnson ZI, Tolonen AC, Rohwer F, Chisholm SW (2004) Transfer of photosynthesis genes to and from *Prochlorococcus* viruses. Proc Natl Acad Sci USA 101: 11013–11018

Long A, McDaniel LD, Mobberley J, Paul JH (2008) Comparison of lysogeny (prophage induction) in heterotrophic bacterial and *Synechococcus* populations in the Gulf of Mexico and Mississippi River plume. ISME J 2:132–144

Lopez D, Kolter R (2010) Extracellular signals that define distinct and coexisting cell fates in *Bacillus subtilis*. FEMS Microbiol Rev 34:134–149

Lopez D, Vlamakis H, Kolter R (2009a) Generation of multiple cell types in *Bacillus subtilis*. FEMS Microbiol Rev 33:152–163

Lopez D, Vlamakis H, Losick R, Kolter R (2009b) Paracrine signaling in a bacterium. Genes Dev 23:1631–1638

Macedo MF, Miller AZ, Dionisio A, Saiz-Jimenez C (2009) Biodiversity of cyanobacteria and green algae on monuments in the Mediterranean Basin: an overview. Microbiology 155:3476–3490

Marenduzzo D, Finan K, Cook PR (2006) The depletion attraction: an underappreciated force driving cellular organization. J Cell Biol 175:681–686

Martinez RE, Pokrovsky OS, Schott J, Oelkers EH (2008) Surface charge and zeta-potential of metabolically active and dead cyanobacteria. J Colloid Interface Sci 323:317–325

Martinez RE, Gardes E, Pokrovsky OS, Schott J, Oelkers EH (2010) Do photosynthetic bacteria have a protective mechanism against carbonate precipitation at their surfaces? Geochim Cosmochim Acta 74:1329–1337

Matei E, Furey W, Gronenborn AM (2008) Solution and crystal structures of a sugar binding site mutant of cyanovirin-N: no evidence of domain swapping. Structure 16:1183–1194

Mateo P, Berrendero E, Perona E, Loza V, Whitton BA (2010) Phosphatase activities of cyanobacteria as indicators of nutrient status in a Pyrenees river. Hydrobiologia 652:255–268

McCarren J, Brahamsha B (2007) SwmB, a 1.12-megadalton protein that is required for nonflagellar swimming motility in *Synechococcus*. J Bacteriol 189:1158–1162

McCarren J, Brahamsha B (2009) Swimming motility mutants of marine *Synechococcus* affected in production and localization of the S-Layer protein SwmA. J Bacteriol 191:1111–1114

McCarren J, Heuser J, Roth R, Yamada N, Martone M, Brahamsha B (2005) Inactivation of swmA results in the loss of an outer cell layer in a swimming *Synechococcus* strain. J Bacteriol 187:224–230

Morsy FM, Kuzuha S, Takani Y, Sakamoto T (2008) Novel thermostable glycosidases in the extracellular matrix of the terrestrial cyanobacterium *Nostoc commune*. J Gen Appl Microbiol 54:243–252

Morvan H, Gloaguen V, Vebret L, Joset F, Hoffmann L (1997) Structure-function investigations on capsular polymers as a necessary step for new biotechnological applications: the case of the cyanobacterium *Mastigocladus laminosus*. Plant Physiol Biochem 35:671–683

Nadell CD, Xavier JB, Foster KR (2009) The sociobiology of biofilms. FEMS Microbiol Rev 33:206–224

Nakao M, Okamoto S, Kohara M, Fujishiro T, Fujisawa T, Sato S et al (2010) CyanoBase: the cyanobacteria genome database update 2010. Nucleic Acids Res 38:D379–D381

Nealson KH, Platt T, Hastings JW (1970) Cellular control of the synthesis and activity of the Bacterial luminescent system. J Bacteriol 104:313–322

OED (2009) Oxford english dictionary. Oxford University Press, Oxford

Paerl HW, Huisman J (2008) Climate. Blooms like it hot. Science 320:57–58

Paerl HW, Steppe TF, Buchan KC, Potts M (2003) Hypersaline cyanobacterial mats as indicators of elevated tropical hurricane activity and associated climate change. Ambio 32:87–90

Peat A, Powell N, Potts M (1988) Ultrastructural analysis of the rehydration of desiccated *Nostoc commune* Hun (Cyanobacteria) with particular reference to the immunolabelling of NifH. Protoplasma 146:72–80

Pereira S, Zille A, Micheletti E, Moradas-Ferreira P, De Philippis R, Tamagnini P (2009) Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev 33:917–941

Pontis HG, Vargas WA, Salerno GL (2007) Structural characterization of the members of a polymer series, compatible solutes in *Anabaena* cells exposed to salt stress. Plant Sci 172:29–35

Potts M (1980) Blue-green-algae (Cyanophyta) in marine coastal environments of the Sinai peninsula: distribution, zonation, stratification, and taxonomic diversity. Phycologia 19:60–73

Potts M (1997) Etymology of the genus name *Nostoc* (cyanobacteria). Int J Syst Bacteriol 47:584

Potts M, Sun H, Mockaitis K, Kennelly PJ, Reed D, Tonks NK (1993) A protein-tyrosine/serine phosphatase encoded by the genome of the cyanobacterium *Nostoc commune* UTEX 584. J Biol Chem 268:7632–7635

Potts M (2000) Nostoc. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 465–504, 669 pp

Rantala A, Fewer DP, Hisbergues M, Rouhiainen L, Vaitomaa J, Börner T (2004) Phylogenetic evidence for the early evolution of microcystin synthesis. Proc Natl Acad Sci USA 101:568–573

Read N, Connell S, Adams DG (2007) Nanoscale visualization of a fibrillar array in the cell wall of filamentous cyanobacteria and its implications for gliding motility. J Bacteriol 189:7361–7366

Redfield RJ (2002) Is quorum sensing a side effect of diffusion sensing? Trends Microbiol 10:365–370

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Romero M, Diggle SP, Heeb S, Camara M, Otero A (2008) Quorum quenching activity in *Anabaena* sp. PCC 7120: identification of AiiC, a novel AHL-acylase. FEMS Microbiol Lett 280:73–80

Romero M, Muro-Pastor AM, Otero A (2011) Quorum sensing *N*-acylhomoserine lactone signals affect nitrogen fixation in the cyanobacterium *Anabaena* sp. PCC7120. FEMS Microbiol Lett 315:101–108

Rycroft CH, Grest GS, Landry JW, Bazant MZ (2006) Analysis of granular flow in a pebble-bed nuclear reactor. Phys Rev E 74:16

Sanchez-Baracaldo P, Hayes PK, Blank CE (2005) Morphological and habitat evolution in the Cyanobacteria using a compartmentalization approach. Geobiology 3:145–165

Sato Y, Okuyama S, Hori K (2007) Primary structure and carbohydrate binding specificity of a potent anti-HIV lectin isolated from the filamentous cyanobacterium *Oscillatoria agardhii*. J Biol Chem 282:11021–11029

Scanlan DJ, Carr NG (1988) Extracellular proteins. In: Packer L, Glazer AN (eds) Methods in enzymology, vol 167. Academic, San Diego, pp 599–605

Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, Post AF, Hagemann M, Paulsen I, Partensky F (2009) Ecological genomics of marine picocyanobacteria. Microbiol Mol Biol Rev 73:249–299

Schaefer AL, Greenberg EP, Oliver CM, Oda Y, Huang JJ, Bittan-Banin G, Peres GM, Schmidt S, Juhaszova K, Sufrin JR et al (2008) A new class of homoserine lactone quorum-sensing signals. Nature 454:595–599

Schatz D, Keren Y, Vardi A, Sukenik A, Carmeli S, Börner T, Dittmann E, Kaplan A et al (2007) Towards clarification of the biological role of microcystins, a family of cyanobacterial toxins. Environ Microbiol 9:965–970

Schaudinn C, Stoodley P, Kainović A, O'Keeffe T, Costerton B, Robinson D, Baum D, Ehrlich G, Webster P (2007) Bacterial biofilms, other structures seen as mainstream concepts. Microbe 2:231–237

Scherer S, Potts M (1989) Novel water-stress protein from a desiccation-tolerant cyanobacterium – purification and partial characterization. J Biol Chem 264:12546–12553

Schirrmeister BE, Antonelli A, Bagheri HC (2011) The origin of multicellularity in cyanobacteria. BMC Evol Biol 11:45

Sergeyenko TV, Los DA (2000) Identification of secreted proteins of the cyanobacterium *Synechocystis* sp strain PCC 6803. FEMS Microbiol Lett 193:213–216

Shahzad-Ul-Hussan S, Cai ML, Bewley CA (2009) Unprecedented glycosidase activity at a lectin carbohydrate-binding site exemplified by the cyanobacterial lectin MVL. J Am Chem Soc 131:16500–16508

Sharif DI, Gallon J, Smith CJ, Dudley E (2008) Quorum sensing in cyanobacteria: N-octanoyl-homoserine lactone release and response, by the epilithic colonial cyanobacterium *Gloeothece* PCC6909. ISME J 2:1171–1182

Shaw E, Hill DR, Brittain N, Wright DJ, Tauber U, Marand H et al (2003) Unusual water flux in the extracellular polysaccharide of the cyanobacterium *Nostoc commune*. Appl Environ Microbiol 69:5679–5684

Shi T, Falkowski PG (2008) Genome evolution in cyanobacteria: the stable core and the variable shell. Proc Natl Acad Sci USA 105:2510–2515

Shin BJ, Oh J, Kang S, Chung YH, Park YM, Kim YH, Kim S, Bhak J, Choi JS (2008) Cyanobacterial hybrid kinase Sll0043 regulates phototaxis by suppressing pilin and twitching motility protein. J Microbiol 46:300–308

Shirkey B, Kovarcik DP, Wright DJ, Wilmoth G, Prickett TF, Helm RF et al (2000) Active Fe-containing superoxide dismutase and abundant sodF mRNA in *Nostoc commune* (cyanobacteria) after years of desiccation. J Bacteriol 182:189–197

Sivonen K, Leikoski N, Fewer DP, Jokela J (2010) Cyanobactins-ribosomal cyclic peptides produced by cyanobacteria. Appl Microbiol Biotechnol 86:1213–1225

Smarda J, Smajs D, Komrska J, Krzyzanek V (2002) S-layers on cell walls of cyanobacteria. Micron 33:257–277

Spence E, Dunlap WC, Shick JM, Long PF (2012) Redundant pathways of sunscreen biosynthesis in a cyanobacterium. Chembiochem 13:531–533

Steinberger RE, Holden PA (2005) Extracellular DNA in single- and multiple-species unsaturated biofilms. Appl Environ Microbiol 71:5404–5410

Stewart PS, Franklin MJ (2008) Physiological heterogeneity in biofilms. Nat Rev Microbiol 6:199–210

Stoodley P, Sauer K, Davies DG, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbiol 56:187–209

Sullivan MB, Lindell D, Lee JA, Thompson LR, Bielawski JP, Chisholm SW (2006) Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. PLoS Biol 4:e234

Sullivan MB, Krastins B, Hughes JL, Kelly L, Chase M, Sarracino D et al (2009) The genome and structural proteome of an ocean siphovirus: a new window into the cyanobacterial 'mobilome'. Environ Microbiol 11:2935–2951

Swingley WD, Blankenship RE, Raymond J (2008) Integrating Markov clustering and molecular phylogenetics to reconstruct the cyanobacterial species tree from conserved protein families. Mol Biol Evol 25:643–654

Timp W, Mirsaidov U, Matsudaira P, Timp G (2009) Jamming prokaryotic cell-to-cell communications in a model biofilm. Lab Chip 9:925–934

Van Wagoner RM, Drummond AK, Wright JLC (2007) Biogenetic diversity of cyanobacterial metabolites. In: Laskin AI, Sariaslani S, Gadd GM (eds) Advances in applied microbiology. Elsevier, Amsterdam, pp 89–217

Ward DM, Cohan FM, Bhaya D, Heidelberg JF, Kuhl M, Grossman A (2008) Genomics, environmental genomics and the issue of microbial species. Heredity 100:207–219

Wase NV, Wright PC (2008) Systems biology of cyanobacterial secondary metabolite production and its role in drug discovery. Expert Opin Drug Discov 3:903–929

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol 21:319–346

Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS (2002) Extracellular DNA required for bacterial biofilm formation. Science 295:1487–1487

Whitehead NA, Barnard AM, Slater H, Simpson NJ, Salmond GP (2001) Quorum-sensing in Gram-negative bacteria. FEMS Microbiol Rev 25:365–404

Whitfield C, Naismith JH (2008) Periplasmic export machines for outer membrane assembly. Curr Opin Struct Biol 18:466–474

Whitton BA, Grainger SLJ, Hawley GRW, Simon JW (1991) Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb Ecol 21:85–98

Whitton BA, Al-Shehri AH, Ellwood NTW, Turner BL (2005) Ecological aspects of phosphatase activity in cyanobacteria, eukaryotic algae and bryophytes. In: Turner BL, Frossard E, Baldwin E (eds) Organic phosphorus in the environment. CABU Publishing, Wallingford/Cambridge, pp 205–241, 399 pp

Wieneke R, Klein S, Geyer A, Loos E (2007) Structural and functional characterization of galactooligosaccharides in *Nostoc commune*: beta-D-galactofuranosyl-(1->6)-[beta-D-galactofuranosyl-(1->6)](2)-beta-D-1,4-anhydrogalactitol and beta-(1->6)-galactofuranosylated homologues. Carbohydr Res 342:2757–2765

Wood SA, Mountfort D, Selwood AI, Holland PT, Puddick J, Cary SC (2008) Widespread distribution and identification of eight novel microcystins in Antarctic cyanobacterial mats. Appl Environ Microbiol 74:7243–7251

Wright DJ, Smith SC, Joardar V, Scherer S, Jervis J, Warren A et al (2005) UV irradiation and desiccation modulate the three-dimensional extracellular matrix of *Nostoc commune* (cyanobacteria). J Biol Chem 280:40271–40281

Wu J, Xi C (2009) Evaluation of different methods for extracting extracellular DNA from the biofilm matrix. Appl Environ Microbiol 75:5390–5395

Yamaguchi M, Ogawa T, Muramoto K, Kamio Y, Jimbo M, Kamiya H (1999) Isolation and characterization of a mannan-binding lectin from the freshwater cyanobacterium (blue-green algae) *Microcystis viridis*. Biochem Biophys Res Commun 265:703–708

Yamasaki Y, Shikata T, Nukata A, Ichiki S, Nagasoe S, Matsubara T et al (2009) Extracellular polysaccharide-protein complexes of a harmful alga mediate the allelopathic control it exerts within the phytoplankton community. ISME J 3:808–817

Yang F, Bewley CA, Louis JM, Gustafson KR, Boyd MR, Gronenborn AM et al (1999) Crystal structure of cyanovirin-N, a potent HIV-inactivating protein, shows unexpected domain swapping. J Mol Biol 288:403–412

Yeager CM, Kornosky JL, Morgan RE, Cain EC, Garcia-Pichel F, Housman DC et al (2007) Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N_2-fixing members of biological soil crusts of the Colorado Plateau, USA. FEMS Microbiol Ecol 60:85–97

Yoon HS, Golden JW (1998) Heterocyst pattern formation controlled by a diffusible peptide. Science 282:935–938

Yoshimura H, Okamoto S, Tsumuraya Y, Ohmori M (2007) Group 3 sigma factor gene, sigJ, a key regulator of desiccation tolerance, regulates the synthesis of extracellular polysaccharide in cyanobacterium *Anabaena* sp. strain PCC 7120. DNA Res 14:13–24

Zhaxybayeva O, Gogarten JP, Charlebois RL, Doolittle WF, Papke RT (2006) Phylogenetic analyses of cyanobacterial genomes: quantification of horizontal gene transfer events. Genome Res 16:1099–1108

Zilliges Y, Kehr J-C, Mikkat S, Bouchier C, Tandeau de Marsac N, Borner T et al (2008) An extracellular glycoprotein is implicated in cell-cell contacts in the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806. J Bacteriol 190:2871–2879

Cyanobacterial Responses to UV Radiation

19

Richard W. Castenholz and Ferran Garcia-Pichel

Contents

R.W. Castenholz (✉)
Institute of Ecology and Evolutionary Biology, University of Oregon, Eugene, OR 97403-5289, USA
e-mail: rcasten@uoregon.edu

F. Garcia-Pichel
School of Life Sciences, Arizona State University, Main Campus, Tempe, AZ 85287-45, USA
e-mail: ferran@asu.edu

Summary

The influence of ultraviolet radiation (UVR) on populations of microorganisms has been the subject of serious investigation for at least the past 20–25 years. UVR that is applicable to the Earth's surface (past or present) is arbitrarily divided into UVA (400–320 or 315 nm), UVB (280–320 or 315 nm), UVC (~180–280 nm). Although essentially all organisms are affected by UVR, microorganisms show more rapid, immediate and measurable effects than macro-organisms. This chapter is mainly relegated to UVR and cyanobacteria, although UV effects on other phototrophs and microorganisms, when relevant, will be included. Some ancestors of living cyanobacteria, the oldest oxygenic organisms, may have evolved in the Archean or early Proterozoic Eons, from 3.5 to 2.5 Gyr, respectively, in a time when UV radiation fluxes reaching the surface, particularly UVB and UVC, were much higher than at present. The latter wavelength region (UVC) does not reach the Earth's surface at present. Thus, cyanobacteria and other microorganisms in that distant age had to have evolved a strategy to tolerate these greater levels of UV radiation, and at present this strategy may demonstrably involve multiple devices, even within one organism. The best understood in the past several years for numerous organisms has been the active metabolic strategies that compensate for the destruction of vital genetic components, such as the development of efficient metabolic DNA repair systems. The implementation of gliding motility system for escaping the effects of high visible and UV radiation has been better described and understood. Some of the most revealing results in the last 10 years have been an almost complete understanding of the regulation of the UV-protective compounds, scytonemin and mycosporine-like compounds, that partially or completely avoid the damage caused by UV radiation.

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19.1 Introduction

Because of the potential to negatively affect molecules and processes of biological importance, ultraviolet radiation (UVR) is considered a detriment to all life forms. There are a few exceptions where UVR may mediate some beneficial responses to high light intensity such as the UVA role in photoreactivation of damaged DNA, and, in some cases, the regulation of some biosynthetic pathways. Although UVR represents only a small proportion of the solar radiation reaching the earth's surface, its effects are inordinately great. There have been several reviews in recent years on the effects of UV radiation (UVR) on microorganisms (e.g. Castenholz and Garcia-Pichel 2000; Vincent and Neale 2000; Whitehead et al. 2000; Roy 2000; Häder 2001; Cockell 2001; Day and Neale 2002; Shick and Dunlap 2002; Hockberger 2002; Castenholz 2004; Singh et al. 2010). This review follows the general pattern of the presentation of Castenholz (2004) and will be focused almost entirely on cyanobacteria. However, when relevant, UVR effects on other organisms will be included. In the natural habitats of cyanobacteria, UVR has a negative effect, particularly during the period of highest solar radiation. If these cyanobacteria are active metabolically, they have the ability to partially ameliorate these negative effects of UVR radiation by various repair mechanisms, often during periods of low light or darkness. Some cyanobacteria that produce the extracellular UV-absorbing compound, scytonemin, may be protected from UVR damage during periods of metabolic inactivity, such as during desiccation or dormancy as a result of suboptimal temperature or freezing (Castenholz and Garcia-Pichel 2000; Castenholz 2004).

Current paleobiological evidence suggests that oxygenic photosynthesis evolved during the early Proterozoic (~2.45–2.32 Gyr, or possibly earlier; Knoll 2008). When the ancestors of cyanobacteria first appeared, the impact of UVR radiation was undoubtedly greater than at present, due to the near lack of O_2 and ozone in the atmosphere, although the luminosity of the sun was diminished during this period. Throughout the Proterozoic, particularly by 2.0 Gyr, cyanobacterial "look-a-like" micro-fossils dominated the presumed photosynthetic picture until about 0.54 Gyr, the end of the Proterozoic (Knoll 2008). Although a high temperature environment has been proposed for the period of early life during the Archean, recent evidence suggests that the Paleoarchean ocean temperatures were no higher than about 40°C (Hren et al. 2009). In this geologic period, before the advent of efficient grazing animals, shallow seas bounding the continental or island land masses were inhabited by cyanobacterial ancestors that formed a variety of microbialites, laminated or not. It is not known when cyanobacteria first invaded terrestrial habitats. However, evidence of microbes in anoxic sediments have been documented as early as 2.75 Gyr instead of the previous suggestion of 1.2 Gyr (Rasmussen et al. 2009). Today there is a much greater variety of both aquatic and terrestrial habitats than in the early Proterozoic or late Archean, and these usually harbour cyanobacteria. Some are exposed to high UVR. UVC (~180–280 nm) was probably quite relevant during these early geologic periods, since it is absorbed by O_2/O_3 in the stratosphere, and only 10^{-5} PAL O_2 (present atmospheric level) was present according to Kasting (1987) and Pavlov and Kasting (2002). This level would not have been sufficient to attenuate UVC or UVB. Alternative conditions that may have resulted in the attenuation of UVR in the primitive anoxic atmosphere may have been sulphur vapor composed of S_8 and other sulfur molecules (Kasting et al. 1989). Also, a high concentration of ferrous ion (Fe II) may have been present in anoxic waters to significantly attenuate UVR (Pierson 1994; Pierson et al. 1993). If no screens of this type were present, 1–4 Wm^{-2} of UVC may have reached the earth and water surface, a value similar to that of maximum intensities of UVB that presently reach the earth's surface (although 5–6 Wm^{-2} may be attained) (Kasting 1987; Cockell 2001). Garcia-Pichel (Fig. 6 in Garcia-Pichel 1998) has estimated the relative damage to a selected cyanobacterium from incoming UVC, UVB, and UVA radiation during the Archean, Proterozoic and Phanerozoic Eons. Cockell (1998) has also estimated the likely biological effects of UVR on early Earth. These estimates show that UVR must have been a very significant evolutionary force during their earliest evolutionary time. Only UVA, the deleterous effects of which are mostly due to indirect, sensitized photoxidative processes, is thought to have intensified as the partial pressure of oxygen increased in the atmosphere (Garcia-Pichel 1998).

This review will summarize the UV tolerance and avoidance strategies known in living cyanobacteria. Some of these adaptive strategies that evolved in cyanobacteria probably did so in the Archean and early Proterozoic, and may now represent relics of that time. The possible influence of UVR fluctuations on plant (and presumably algal and cyanobacterial evolution), particularly during the Cenozoic have been discussed by Willis et al. (2009). A few passive "strategies", include burial at shallow sediments, growth at greater water depths (avoidance), and the seemingly altruistic sacrifice of surface layers in microbial mats, will not be discussed here. These are forms of relegation to refugia, that imply the loss of potential habitat. Instead, biochemical aspects of tolerance, UV shielding and behavioural escape mechanisms will be discussed here. This includes recently elaborated signal responses, and biochemical pathways of mycosporine-like amino acid (MAA) and scytonemin induction and synthesis. Although it is difficult to discuss the effects and responses to the three arbitrarily defined categories of UVR (UVC, UVB, UVA), an attempt will be made to present this review in that fashion. However, at first, we will describe the habitats in which cyanobacteria are most at risk to UV exposure. The readers are referred to Castenholz and Garcia-Pichel (2000) for topics not included here.

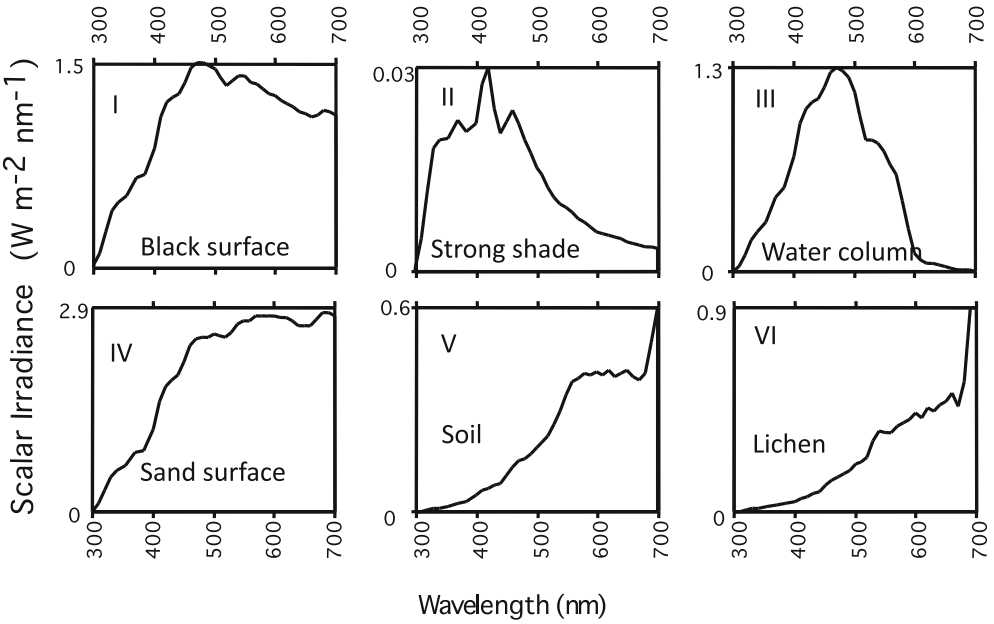

Fig. 19.1 Effects of habitat on the exposure of cyanobacteria to solar radiation: (**I**) Spectral scalar irradiance (sun and sky) incident at ground level at noon during a clear midsummer day at 41°N. Lat. (**II**) "Strong shade" habitat (N. facing surface illuminated by very diffuse sky radiation only). Although scalar irradiance is very low, the relative importance of UVR is enhanced. (**III**) Open ocean habitat (under 1 m of clear water) where all fluxes remain fairly high and UVB and visible light are more strongly attenuated than UVA. (**IV**) Surface of beach sand (quartz and feldspar) where all UVB, UVA and visible are higher than incident (by 120%, 150% and 205%, respectively) due to light trapping effects. (**V**) 300 μm deep in a wet topsoil where UVB and UVA have been attenuated below 5% of incident, but ca. 20% of visible light remains. (**VI**) Scalar irradiance within the thallus of the terrestrial cyanobacterial lichen *Collema* sp. (From Fig. 1 in Castenholz and Garcia-Pichel 2000, with references. With kind permission from Springer Science + Business Media B.V.)

19.2 UVR Exposure in Natural Habitats

Latitude determines to a great extent the flux of UVR incident at ground level, the maximum instantaneous and yearly dosage, as well as the amplitude of seasonal variation. Large seasonal variation and low maximal values are associated with higher latitudes, whereas strong, seasonal irradiances correspond to lower latitudes. High altitude, by decreasing the semi-spherical path length for atmospheric attenuation, also results in higher solar radiation fluxes. Other meteorological factors may modify the incident UVR, such as cloud cover, air pollution, and the optical thickness of the stratosphere that is regionally altered by ozone depletion (Roy et al. 1994). Industrial air pollutants, such as sulphur dioxide, nitrogen dioxide and soot particles may reduce incoming UVR by as much as 20%, thus, offsetting the potential effect of ozone depletion (Kvalevåg et al. 2009). Direct measurements of UVR are obviously preferred, but irradiance data are available from various meteorological institutions, although UVR information is often missing (Castenholz and Garcia-Pichel 2000). Total solar radiation, and the UVA and UVB components have been monitored for over 10 years in Europe and some other locations including stations in the Southern Hemisphere (see Häder et al. 2007). The measurement of UVR reaching ground level is not entirely indicative of any organism's exposure. Absorptive and scattering properties within the micro-environment of the organism may greatly modify the simple assessment of the scalar irradiance as measured by a biospherical photon collection instrument (Fig. 19.1). These modifications are not always intuitively evident or easy to measure.

Three types of "optical" habitats are first considered: planktonic, benthic, and terrestrial. Exposure in planktonic habitats is determined by the attenuation rate within the water column and by the mixing regime of the water mass. Typically, cells are subjected to high and low exposure periods as they are brought up and down by turbulence (Cullen and Neale 1994). A time integration of UV exposure per cell is very difficult to calculate in these circumstances because of complex hydrodynamic mixing processes (Castenholz and Garcia-Pichel 2000). A comparison of several natural marine waters showed that the UV doses received by cells in the euphotic zone ranged between 3% and 9% of the incident doses that would be received if they remained at the surface (Garcia-Pichel and Bebout 1996). Ultra-oligotrophic lake and most oceanic seawater are quite transparent to UVR. A detailed re-evaluation of visible and UV radiation penetration in the clearest oceanic waters was presented by Morel et al. (2007). UVA can penetrate to nearly the maximum depth of the euphotic zone (Smith and Baker 1981; Helbling et al. 1994; Prezelin et al. 1994). UVB is attenuated

more steeply, but still a low intensity may reach the limit of the euphotic depth. Turbidity, of course, will greatly reduce UVR penetration, but more selectively dissolved organic matter (DOM), particularly humic acids, a general term for coloured organic solutes that are generally derived from vegetation, especially from littoral and terrestrial sources in lakes, ponds, and river backwaters, and from near shore macroalgae in the marine environment, will have the greatest impact. (Vincent and Roy 1993; Scully et al. 1996; Karlsson et al. 2009). In many lakes, particularly during summer stratification when cyanobacterial and algal populations may be vertically stabilized in the metalimnion or upper portion of the hypolimnion, dense populations of cyanobacteria may accumulate. This is conspicuous in eutrophic and mesotrophic lakes in which dim green light zones are dominated by gas-vesiculate filamentous cyanobacteria, red in colour due to a high cell content of phycoerythrin. However, little UVR is likely to reach such depths in these waters. In terrestrial habitats, including shallow water springs and streams, ephemeral slacks, and moist surfaces, two factors locally influence the incident UVR, the orientation of the surface with respect to the solar vertical, and the albedo of the surface. Instantaneous exposure and dose are highest on surfaces oriented orthogonally to the sun's vertical, and lowest in those in the optical shadow of solids (Fig. 19.1). However, due to the atmospheric (Rayleigh) preferential scatter of the shorter wavelengths, the spectral composition of the diffuse light that penetrates the shade behind solids is highly enriched in UVR (Robinson 1966; Frederick et al. 1989). At the wavelength of 300 nm (UVB), approximately half of the incident radiation is diffuse and half comes directly from the solar disc. Thus, populations thriving on shaded surfaces will receive higher UVR doses relative to the visible than populations on sunny surfaces, but with lower absolute doses of UVR. The implication then is that UVR may play an important role for cyanobacteria exposed to diffuse light. The radiation reflected from the surface may change the absolute and spectral characteristics of the absorbed radiation with respect to the incident radiation. For example, substrates such as white sandstone and carbonaceous materials (e.g. travertine, other limestones, coral sand, and concrete) reflect UVR strongly (Koller 1965; Diffey et al. 1995). In contrast, sedimentary environments (including microbial mats) are characterized by the strong attenuation of both visible and UVR due to absorption and multiple scattering by the matrix and the organisms, so that phototrophic metabolism is restricted to thin (millimeter to centimeter) surface layers (e.g. Castenholz and Garcia-Pichel 2000; Kruschel and Castenholz 1998; Garcia-Pichel et al. 1994). When close to the surface, light-trapping phenomena result in localized irradiance radiation maxima that are higher than the incidence radiation. Typically, then, there is an onset of quasi-exponential attenuation of light below the light-trapping. Thus, in sedimentary habitats, extremely steep gradients of exposure ranging from zones of increased exposure to zones that are refuges from UVR. This condition also applies to endolithic populations in many habitats. Because light-trapping effects are greater at longer wavelengths, and absorption by particulates is most pronounced at shorter wavelengths, the ratio of UVR to visible invariably decreases with depth. A comparison of data from several sedimentary micro-environments showed that the space-averaged UVR dosage rates within the euphotic depth ranged from 15% to 33% of the incident value, a much higher UVR exposure than that in the euphotic zones of water columns (Garcia-Pichel and Bebout 1996).

A recent experiment that placed endo- and epilithic intertidal microorganisms into low orbit space at a height of about 300 km for 10 days that included exposure to vacuum, desiccation, and high intensity UVR resulted in the survival of a coccoid, aggregated cyanobacterium (Olsson-Francis et al. 2010). However, there was no information on the intensity of UVR that actually reached the endolithic cyanobacteria.

Other aspects of biologically effective UVR exposure, such as biological weighing functions are discussed in Castenholz and Garcia-Pichel (2000).

19.3 Metabolically – Timed Exposure

Cyanobacteria are often the predominant phototrophs in habitats where metabolic activity is restricted due to lack of water or nutrients, freezing or sub-optimal temperatures. Under conditions of restricted or interrupted active metabolism, the impact of exposure to solar radiation may seriously exceed that predicted by simply expressing exposure in terms of total time. Dose modification factors based on the fraction of total time that the organisms are active, can be used to obtain a more relevant metabolically-timed exposure. This may be relatively easy where the time partition is clear but quite complicated if metabolism slows rather than halts. An extreme example may be the surface-dwelling soil cyanobacteria of the Colorado Plateau (e.g. *Nostoc*, *Scytonema*, *Microcoleus*). These populations may experience about 100 h of wetness (active metabolism) during the winter season and additional scattered periods during and after summer rains (Garcia-Pichel and Belnap 1996). Approximately one half of the periods of wetness occur during daytime. However, the populations are exposed to UVR throughout the year. Thus, the UVR damage accumulated during a year's exposure needs to be rectified by repair and other metabolic processes within the possible 120 h when cells are potentially active. This represents a metabolically-timed dosage of about 70 times the actual incident dose. This metabolically-timed irradiance is likely to be a better predictive factor in the terrestrial habitat of desert crusts, in polar mats, and in alpine situations, where desiccation and/or cold are important factors. However, all of these types of theoretical calculations are nullified if the

organisms have an extracellular UVR-protectant, such as the sheath "pigment", scytonemin, or layers of dust/soil that would prevent much of the UVR from reaching the cell interior.

19.4 Examples of Cyanobacterial Habitats with High Exposure

The Proterozoic Eon (2.5–0.54 Gyr) was populated by cyanobacteria or their oxygenic ancestors, as demonstrated by micro-fossil and relic chemical evidence as well as indirectly by the early oxygenation of the waters and atmosphere (Knoll 2008). In this geologic period, before the advent of efficient grazing animals, shallow seas bounding the continental or island land masses were inhabited by cyanobacterial ancestors that resulted in the formation of a variety of stromatolites. Today there is a much greater variety of both aquatic and terrestrial habitats and niches than in the early Proterozoic or late Archean, and these usually harbour cyanobacteria. Some are exposed to high UVR.

19.4.1 Hot Springs

Although hot springs occur today in many locations associated either with volcanic activity (residual or current) or tectonic activity, most terrestrial springs are exposed to full sun because vegetation near them is usually low or absent due to high soil temperatures, and possibly toxic compounds such as arsenite and arsenate. High elevation is another factor that exposes such habitats to high levels of solar irradiance. This was pointed out by Phoenix et al. (2006) who studied hot spring cyanobacterial habitats at over 5,000 m elevation in Bolivia. However, the UVR fluxes measured (perhaps incorrectly) were no higher than those at the major thermal areas of Yellowstone National Park, most of which are close to 2,500 m. The measurements in Bolivia were also quite comparable to midday values in Argentina, some at sea level, but during a low ozone period (Orce and Helbling 1997). In Iceland, many geothermal springs occur only slightly above sea level, but no trees or shading vegetation occurs, and high levels of UVR should be expected. However, the solar angle in summer at over 65°N. reduces total solar intensity, but not necessarily 24 h dosage during the summer months. Many or most hot springs produce shallow runoffs of clear water that flows over microbial mats that are topped by cyanobacteria or simply biofilms of cyanobacteria at the upper end of their thermal range for growth (72–73°C), the highest known temperature for photosynthesis (Fig. 19.2) This limit applies to some areas in the western US and parts of southeastern Asia. However, the upper temperature limit is considerably lower (63–64°C) in other regions (including New Zealand, Iceland and Europe) (see Ward and Castenholz 2000) due to the absence of the highest temperature form of *Synechococcus* in these and other regions. Metabolic and carotenoid mechanisms for UVR damage repair occur in probably all cyanobacteria (Fig. 19.3) When hot spring runoff reaches average temperatures below about 50–55°C many cyanobacteria also produce scytonemin, an extracellular sheath UVR-shielding

Fig. 19.2 An alkaline hot spring in the Lower Geyser Basin, YNP ("Five Sisters") in summer showing upper temperature limit (70–73°C) of *Synechococcus* sp.: *yellow* cover on *left* and foreground, rich in carotenoids; *Synechococcus* sp. also in *deep shaded* basin, rich in chlorophyll and phycocyanin. *Gray* area is covered with water (>74°C), originating from spring source in *upper left*. *Dark* moist crust along *left* and *right* edge consists of various cyanobacteria containing scytonemin (Photo by RWC)

Fig. 19.3 Alkaline White Creek, Lower Geyser Basin, YNP, with a mean temperature at this point of 50–55°C, with green trailing wisps of *Mastigocladus* (=*Fischerella*), *Leptolyngbya* and *Synechococcus*, the last attached mainly to streamers of *Chloroflexus*-like anoxygenic bacteria. The *rich green* of the cyanobacteria in the fast-flowing stream is probably a result of continuous waving motion of the streamers, thus lessening constant exposure to high solar radiation. The *orange* static film of cyanobacteria fanning down the embankment is rich in carotenoids, probably because of suboptimal temperature and high solar irradiance that results in a high carotenoid to chlorophyll + phycocyanin ratio (Photo by RWC)

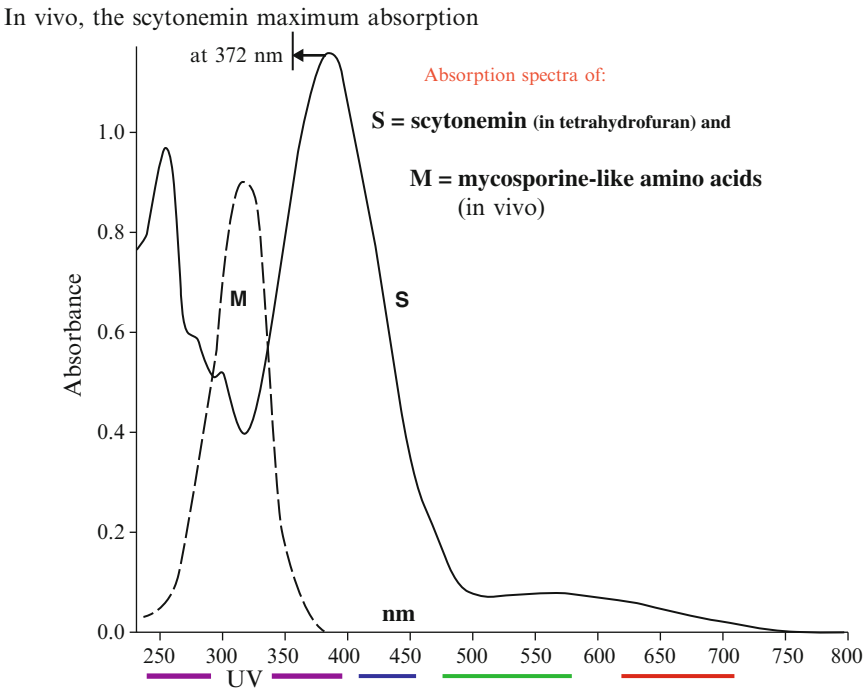

Fig. 19.4 Absorption spectrum of scytonemin in tetrahydrofuran. The *in vivo* maximum absorption peak is at 372 nm. The spectrum of a generalized MAA has been added, as well as colour guides to nm ranges (Modified from Proteau et al. 1993. With kind permission from Springer Science + Business Media B.V.)

Fig. 19.5 Alkaline Grand Prismatic Spring in Midway Geyser Basin, YNP. High temperature *Synechococcus* sp. in distance (*greenish* at edge of pool). *Orange* flow in shallow water from ~60°C to ~50°C consisting mainly of *Leptolyngbya* spp. (=*Phormidium*) and *Synechococcus* spp. The cells are rich in carotenoids and low in chlorophyll and phycocyanin content. The *brown* band (<50°C) consists mainly of *Calothrix* sp. with a high content of scytonemin in the filament sheaths (Photo by RWC)

pigment that may actually prevent damage even when the cells are metabolically inactive (Fig. 19.4). The scytonemin-producing cyanobacterial species in Yellowstone (e.g. *Calothrix, Scytonema* and *Nostoc*) are those that grow only below ~45–50°C (Fig. 19.5). It has also been shown that acidic hot springs (pH 0.5–4.0, ~35–56°C) harbour only one type of phototroph, namely the unicellular members of the Cyanidiales (Rhodophyta). In their natural shallow stream habitats they are significantly inhibited by high solar radiation, particularly UVR (Lehr et al. 2007).

19.4.2 Intertidal Marine and Hypersaline Habitats

Intertidal marine habitats close to "normal" seawater salinity (~31–35 ppt), such as mud flats and salt-water marshes are usually devoid of perennial cyanobacterial mats, probably because of an abundance of invertebrate herbivores and algal and plant competitors. Nevertheless, there are some tidal flats covered by water mainly during spring tides, and these exclude most grazing invertebrates because of desiccation and high salt intolerance as the flats dry out. Some of these flats (e.g. Laguna Guerrero Negro, Baja California Sur, Mexico) are dominated by scytonemin-producing cyanobacteria (*Lyngbya* cf. *aestuarii* and *Calothrix* sp.). These almost monospecific mats inhabit the mid-intertidal (*Lyngbya*), and uppermost intertidal (*Calothrix*) and cover vast areas in this location (Javor and Castenholz 1981, 1984). Both these cyanobacteria in nature contain scytonemin in their sheaths (Fig. 19.6). Other intertidal sedimentary habitats, such as the Great Sippewissett Marsh, Massachusetts, contain so much biogenic sulfide in the sediments that many grazers are absent or restricted, and highly exposed and cyanobacterial mats develop mainly in the summer. The benthic mats of shallow, natural or man-made, hypersaline pools and lagoons also

Fig. 19.6 Intertidal flat at Laguna Ojo de Liebre, Baja California Sur, Mexico. The *dark area* is covered by the scytonemin-rich cyanobacterium, *Lyngbya* cf. *aestuarii* during neap tide. This area is covered by seawater of 35–50‰ salinity during periods of spring tides (~every 2 weeks) (From Castenholz 2009, Mats/microbial, in encyclopedia of microbiology, Fig. 9, p 287. With kind permission of Elsevier, Inc.)

Fig. 19.7 *Stigonema* sp. from 1 m depth in ultra-oligotrophic Waldo Lake, Oregon Cascade Mountains: *Yellow* colour of sheath is caused by scytonemin. Diameter of main axis is ~7 μm (Photo by RWC)

provide habitats with high insolation where cyanobacteria usually dominate, at least at salinities above 50–60 ppt, a level that usually excludes grazing herbivores except for harpactipoid copepods, nematodes, and various "protozoa" (Des Marais 1995; Garcia-Pichel et al. 1994; Kruschel and Castenholz 1998). In warm, clear tropical waters, several types of cyanobacteria, including living stromatolite-like cushions, and both filamentous and gelatinous cyanobacterial tufts and epilithic biofilms attached to sediment, rocks, or macro-algae are exposed to high levels of UVR (Castenholz and Garcia-Pichel 2000).

19.4.3 Benthic Freshwater Habitats

Clear water lakes and streams are common in many areas distant from human populations. These include a large percentage of oligotrophic alpine/subalpine and polar lakes and ponds (Vinebrooke and Leavitt 1996; Vincent 2000). Although many of these habitats are not dominated by cyanobacteria, there are various algae that are typical. However, in ultra-oligotrophic lakes and ponds in polar regions cyanobacterial mats again become predominant, possibly because of the lesser ability of many or most eukaryotic algae to tolerate long- or short-term and frequent freezing and thawing (Vincent et al. 1993, 2004; Tang et al. 1997; Bonilla et al. 2005) and also, in some cases, because of the low herbivore diversity and density. One ultra-oligotrophic lake in the Oregon Cascades (Waldo Lake) that is essentially equivalent to distilled water in terms of solutes, is dominated by benthic populations of cyanobacterial populations of the genera, *Stigonema* and *Scytonema*, that have sheaths rich in scytonemin to depths of at least 15 m (Johnson and Castenholz 2000) (Figs. 19.4 and 19.7). These oligotrophic waters are dominated by heterocystous cyanobacteria by virtue of an extreme deficiency of combined nitrogen. Another example of this is the slow-growing giant *Nostoc* cf. *pruniforme*, forming scytonemin-containing colonies that form an almost monospecific population in a few cold water spring-fed ponds in southern Oregon that never exceed ~5°C (Dodds and Castenholz 1988; photo in Castenholz and Garcia-Pichel 2000). Although dominance by cyanobacteria in cold waters would have been unexpected some years ago, Antarctic and Arctic freshwater and saline ponds and lakes are nevertheless dominated by cyanobacterial mats (Vincent 1988; Tang et al. 1997). It was long thought that cyanobacteria were more adapted to warmer waters. It appears, however, that most of the cyanobacterial species in these habitats are simply more tolerant of cold and freezing than most eukaryotic algae, although their optimal temperature for photosynthesis and growth may be considerably higher than in their ambient cold water habitats (Nadeau and Castenholz 2000; Castenholz and Schneider 1993). Heterocystous *Nostoc* with scytonemin is very common in glacial meltwater streams and ponds in the Antarctic dry valleys (Vincent 1988; Castenholz, unpublished observations).

19.4.4 Marine and Freshwater Plankton

Although much information is available on the effects of UVR on marine, planktonic diatoms, and various photosynthetic flagellates (Gieskes and Buma 1997; Bouchard et al. 2005; Sobrino et al. 2008; Laurion and Roy 2009), there is little information on the specific effects of UVR on marine

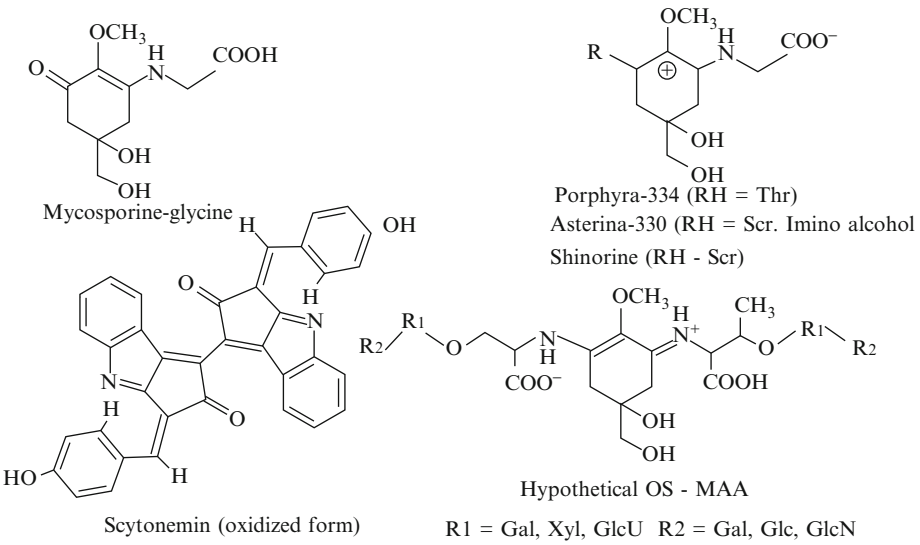

Fig. 19.8 Chemical structure of some compounds in cyanobacteria involved in UVR protection, including scytonemin and 5 types of mycosporine-like amino acids. OS-MAA is found in the EPS of *Nostoc* cf. *commune* (Ehling-Schulz et al. 1997) (From Fig. 2 in Castenholz and Garcia-Pichel 2000, with other references. With kind permission from Springer Science + Business Media B.V.)

cyano-plankton. The filamentous gas-vesiculate *Trichodesmium*, the principal N_2-fixer of warmer, nitrate-depleted oceanic waters, is subject to the production of high amounts of reactive oxygen species, including oxygen radicals due to the impact of high visible and UV radiation. It produces the highly potent antioxidant, all-trans-ß-carotene that may counter the detrimental effects of reactive oxygen (Kelman et al. 2009). *Trichodesmium* and some other marine and freshwater planktonic cyanobacteria are known to accumulate large quantities of MAAs (mycosporine-like amino acids) that are, in these cases, intracellular UVR absorbers (Fig. 19.8). However, MAAs apparently do not occur in small unicellular planktonic cyanobacteria (Garcia-Pichel and Appel, unpublished data). Meador et al. (2009) demonstrated UVR-DNA damage in surface dwelling microorganisms over a long latitudinal range (70°N–68°S in the Pacific with some consideration of the picoplanktonic cyanobacteria, *Synechococcus* and *Prochlorococcus*. UVR may penetrate to considerable depth in clear oceanic waters; in some cases UVB to over 30 m and UVA in excess of 60 m (Holm-Hansen et al. 1993; Jeffrey et al. 1996; Booth and Morrow 1997; Morel et al. 2007). However, in coastal waters UVR (and especially UVB) may show a rapid attenuation to <1% in less than 0.5 m depth (Nielsen and Ekelund 1995).

In general, high-altitude lakes are not dominated by cyanobacteria, but although cyanobacteria (*Synechococcus* sp.) predominated in several lakes of the Tibetan Plateau (~3,200–4,700 m elevation), a low taxon richness was evident (Xing et al. 2009). In any case, most subalpine and alpine lakes and ponds contain clear water and thus, have high exposures to solar irradiance. Freshwater unicellular cyanobacteria of pico-planktonic size also occur in many of these waters (Weisse 1993; Eguchi et al. 1996; Postius et al. 1996). In many temperate lakes, especially eutrophic types, gas-vacuolate cyanobacteria often predominate (e.g. *Aphanizomenon, Anabaena, Planktothrix, Limnothrix, Microcystis*) and may rise to the surface during periods of low wind and are destroyed by high solar irradiance. During such a calm period in sunlit Upper Klamath Lake, Oregon , there was there was an almost complete extermination (only phycocyanin remained) of dense *Aphanizomenon* populations that floated to the surface (Castenholz, unpublished observations).

19.4.5 Terrestrial Habitats

In exposed terrestrial habitats that are often extreme by virtue of long-term or periodic desiccation, many species of cyanobacteria thrive because of their tolerance to desiccation, but also because of a high tolerance, in many cases, to UVR (Potts 1994). The terrestrial cyanobacterial mats or crusts (often known as biological soil crusts) occur in many of the warm and cold deserts of the earth, where these may form an extensive ground cover when undisturbed by human practices, and sometimes surrounding protective desert shrubs (Garcia-Pichel and Belnap 1996; Mazor et al. 1996). The harder substrates, such as rocks and cliff faces are often covered with thin dark covers of epilithic cyanobacteria ("tintenstriche") that by virtue of their unprotected location, obviously receive much UVR whether wet or dry. Most of these cyanobacteria are dark in colour because of sheaths or EPS that contain scytonemin. These generally do not occur on the most sun-exposed faces which

are often too desiccated to allow any free-living phototrophs, except for lichens, some of which are inhabited by cyanobacterial photobionts that also contain scytonemin (Büdel et al. 1997). The well-known *Nostoc flagelliforme* of semi-desert regions appears to be insensitive to UVR under both desiccated and rehydrated conditions (Gao and Ye 2007). There is a large literature on endolithic and chasmolithic cyanobacteria within limestone (including travertine), dolomite, sandstone, and other somewhat porous rocks (Norris and Castenholz 2006). Most of these situations involve a narrow band (1–2 mm thick) 1–3 mm below the rock surface. Although only one publication has measured light penetration to the level of the phototroph band (Matthes et al. 2001), it is assumed that the specific depth of the chlorophyll band represents many strategies, such as avoidance of high light and UVR, as well as optimization of the light intensity, moisture retention, and prevention of erosion by wind. Such endolithic populations are known world-wide in hot and cold, deserts (e.g. Nienow and Friedmann 1993) as well as in rocks exposed to seasonal desiccation, wetting, and freezing, such as in travertine in Yellowstone National Park and the Rocky Mountains (Norris and Castenholz 2006; Walker and Pace 2007). Some isolates of the desiccation tolerant *Chroococcidiopsis* (a unicellular cyanobacterium) are also capable of tolerating up to 5 kGy of ionizing radiation (X-rays), while desiccated, and may be comparable to the tolerance and DNA repair mechanisms of *Deinococcus,* a heterotrophic bacterium well-known for this ability (Billi et al. 2000).

19.5 Negative Effects of UVR and Physiological and Biochemical Strategies of Counterbalance

The targets of damage by UVR with respect to metabolism, DNA, development, and behaviour for cyanobacteria were summarized in Table 1 in Castenholz and Garcia-Pichel (2000), while studies on the effects of UVR on the morphology of filamentous cyanobacteria have been reported by Wu et al. (2005) and Gao et al. (2008). Summaries of the effects of UVR on other organisms were summarized by Jägger (1985), Häder (2001), and Hockberger (2002). The number of targets and detrimental effects mount exponentially with shortening wavelengths (Jägger 1985; Whitehead et al. 2000). However, under standard conditions of environmental exposure, UVC is no longer environmentally relevant, because of complete atmospheric absorption. Since UVC and UVB radiation have the most lethal effects, and because these spectral regions reached the Earth's surface without atmospheric interference only in the Archean and early Proterozoic, they will be discussed first.

19.5.1 UVC and UVB

Germicidal lamps that emit in the UVC range have been used in many experiments with bacteria, viruses, DNA and proteins (see Jägger 1985). Damage is mainly through direct absorption by DNA. DNA absorbs in three spectral regions: I (with a peak at 260–264 nm), II (with a peak at 192 nm), and III (below 125 nm). Spectral regions II and III would only have relevance in outer space (see Zalar et al. 2007a, b; Olsson-Francis et al. 2010). The effect of UVC radiation (254 nm max) negatively affected short-term photosynthetic rate and survival in *Agmenellum quadruplicatum* (now *Synechococcus* sp. PCC 73109)(Van Baalen 1968). However, the damage to DNA was cured by photoreactivation at wavelengths from ~395 to 450 nm (Van Baalen and O'Donnell 1972).

Chloroflexus aurantiacus, a primarily photoheterotrophic anoxygenic bacterium, showed relatively high UVC-tolerance which may have had relevance during the Archean when the Chloroflexi may have evolved (Pierson et al. 1993). Results of continuous UVC radiation within the possible Archean limits, resulted in yields similar to those of controls under anoxic conditions at 0.01 Wm^{-2} UVC, an intensity that severely inhibited *E. coli.* This exposure could not occur indefinitely without DNA damage and deleterious accumulation of mutations. Pierson et al. (1993) suggested that the Archean sediments may have carried a load of ferric and ferrous ions that blocked UV wavelengths, but still passed the longer visible wavelengths that support photosynthesis.

Cyanobacteria that inhabit exposed, near-surface habitats usually synthesize the UVR-absorbing pigment, scytonemin, in their sheaths or EPS. This compound has major absorption maxima in the UVC and UVA spectral regions, but also ample absorption in the UVB (Proteau et al. 1993) (Fig. 19.4). During the early Precambrian an extracellular compound that absorbed UVC may have been of considerable benefit. O_2 is a requirement for scytonemin synthesis, but internal O_2 release as a product of oxygenic photosynthesis must have occurred long before the general oxygenation of the waters. Although scytonemin functions primarily today as a UVA and UVB screen, Dillon and Castenholz (1999) found that it afforded considerable protection against UVC photosynthetic damage in scytonemin-containing *Calothrix* and *Chroococcidiopsis* cultures when 0.5–1.0 Wm^{-2} UVC radiation was added to natural solar irradiance. Many intracellular and extracellular compounds absorb in the UVC region, however, the presence of scytonemin in the external sheath provided protection that the glycan sheath without scytonemin did not.

UVB is less potent energetically than UVC, but the type of damage is similar. UVB includes the wavelengths that are most harmful to humans by causing sunburn and skin cancer (e.g. melanoma). The recent increase in UVB in some regions

is due to periodic stratospheric losses of ozone. The UVB (280–315/320 nm) intensity reaching the earth's surface is sufficient to cause damage to many cellular components in micro-and macro-organisms, including DNA as a direct target. The result is the formation of various photoproducts (e.g., cyclobutane-pyrimidine dimers, pyrimidine (6–4) pyrimidone products, and cytosine products) (Ravanat et al. 2001). Purine bases are also photoreactive. Other impairments include accelerated degradation of photosystem II proteins (e.g. D1/D2) and destruction of the light-harvesting phycobiliproteins, since these have a small absorbance peak in the UVB (Lao and Glazer 1996; Wingard et al. 1997). Also, various activities such as synthesis of chlorophyll, energy transfer in light harvesting, nitrogen fixation, RUBISCO and ATP synthase activities, nitrate and ammonium uptake, nitrogen fixation, motility and photoorientation, and cell differentiation are negatively affected by UVB radiation (Häder 1984; Sass et al. 1997; Choi et al. 1999; Castenholz and Garcia-Pichel 2000). UVB damage may be more severe under nutrient limitation. Damage to light-harvesting complexes in cyanobacteria may exceed the damage to DNA in some cases (Lao and Glazer 1996). D1/D2 proteins of PS II reaction center may be destroyed not only directly by UVR but also indirectly by reactive oxygen produced by high intensity violet/blue light or UV radiation. Rapid *de novo* synthesis of D1/D2 proteins is an essential part of the repair process (Sass et al. 1997). Several proteins may be photooxidized by UVB radiation since tryptophan, tyrosine, phenylalanine and histidine absorb in the 290–315 nm range (MacDonald et al. 2003). In one case, photooxidative inhibition and damage by moderate UVB was reversed after about 2 weeks of continuous exposure in a species of *Anabaena* (He et al. 2002). MacDonald et al. (2003) found that a naturally high ratio of visible light to UVB resulted in a greater tolerance to UVB in *Synechococcus* PCC 7942.

The exposure of the same unicellular cyanobacterium (*Anacystis nidulans* R-2 = *Synechococcus* PC 7942) to UVB and UVA resulted in the production of several "UV-shock" proteins, some of which may function as enzymes that scavenge reactive oxygen molecules (Shibata et al. 1991). Earlier, the induction of the synthesis of a few specific proteins in thermophilic *Phormidium* cf. *laminosum* was reported in response to inhibitory UV radiation (Nicholson et al. 1987). Another study, again using *Synechococcus* PCC 7942, has demonstrated that an ATP-dependent Clp protease is essential for acclimation to UVB, and results in the rise of a modified D1 PS II protein (D1:2) that is more resistant to UVB stress (Porankiewicz et al. 1998). The *psbA* genes (*psbA2* and *psbA3*) that code for a more UVB tolerant, PS II D1 protein may be activated in *Synechocystis* PCC 6803 under even low levels of UVB exposure (Máté et al. 1998; Campbell et al. 1998). After 2 h exposure of *Synechocystis* PCC 6803 to 20 μE m^{-2} s^{-1} UVB the levels of expression of 55 genes rose more than twofold and those of 44 genes fell more than twofold (Los et al. 2008). With whole genome profiling of some phototrophic prokaryotes now possible, transcripts from genes involved in various light (or UVR) intensity transitions can be probed with full genome DNA microarrays (see Gill et al. 2002; Los et al. 2008).

UVB affects shallow benthic phototrophs, (e.g. in intertidal flats, shallow lagoons and lakes), also phytoplankton, especially in clear oceanic waters where harmful intensities may reach over 20 m (much less in turbid or waters rich in coloured dissolved organic matter), as well as exposed terrestrial crusts and epi- and endo-lithic populations of cyanobacteria and microalgae. There is a particularly large literature on the effects of UVR (mainly UVB) on phytoplankton which often includes diatoms, dinoflagellates, and other micro-algae, but there is little specific information on the well described cyanobacterial picoplankton, such as *Synechococcus*, *Prochlorococcus* and the less well-known *Crocosphaera.* Effects of UVB in lakes and the sea are particularly difficult to assess because of vertical and horizontal mixing and, thus, like other field studies, requires extrapolation of short term daylight results to full days, weeks and seasons (Day and Neale 2002). Nevertheless, work in the Southern Ocean by various investigators have estimated that phytoplankton productivity has been reduced by ~0.2–2.4% as a result of increased UVB that was presumably due to periodic ozone depletion. However, estimates of this type differ greatly (see Day and Neale 2002 for references). As with UVC, some of the negative effects on DNA (formation of cyclobutane pyrimidine dimers and 6–4 photoproducts that block DNA and RNA polymerases) may be repaired by photoreactivation, utilizing UVA and/or violet/blue wavelengths that are absorbed by chromophores of photolyase, a DNA-bound enzyme that restores the intact bases, and also by nucleotide excision repair (Jägger 1985; Sancar 1994, 1996). Increases in temperature have been shown to enhance the DNA photoreactivation repair rates in algae (Pakker et al. 2000).

In an extensive review, Bailey and Grossman (2008) have discussed the evidence that a photoprotective method of dissipating excess photon (quantum) energy by the light-harvesting complex is by down-regulating the transfer of this excitation to the photosynthetic reaction centres. This is termed non-photochemical quenching (NPQ), and the authors have concluded that this process also occurs in cyanobacteria although only previously known in algae and plants. NPQ in cyanobacteria appears to involve the absorption of blue light by a carotenoid-binding protein and probably involves quenching in the phycobilisome core. Although these reactions are known only for the short wavelength visible spectrum, it is possible that excess UVR absorption may be dissipated by a NPQ quenching reaction as well.

Sytonemin has substantial absorbance in the UVB spectral region. However, the degree of protection afforded specifically by scytonemin has not been tested in this spectral region (Fig. 19.4). It may, however, be insufficient, as most scytonemin synthesizers complement their sunscreen capabilities with mycosporine-like amino acids (MAAs), Mycosporine-like amino acid derivatives (MAAs) are known in very many cyanobacteria (Garcia-Pichel and Castenholz 1993), numerous microalgae, and marine zooplankton (Castenholz and Garcia-Pichel 2000; Shick and Dunlap 2002). MAAs are low molecular weight, water soluble compounds that are condensation derivatives of a cyclohexenone ring and amino acid (or imino alcohol residues), but are acquired secondarily by invertebrates through consumption of primary producers (Fig. 19.8) Radiotracer experiments have shown that in cyanobacteria the core cyclohexenone is derived from the shikimate pathway, and amino acids are condensed onto it directly as precursors (Portwich and Garcia-Pichel 2003). Gadusols, that are putative intermediates in the shikimate pathway of MAA synthesis, may have been early absorbers of UVC/B radiation and may also have served as strong antioxidants (Shick and Dunlap 2002). The absorption peaks of MAAs range from 310 to 360 nm (i.e. from UVB into the UVA) (Fig. 19.4). Short wavelength MAAs are monosubstituted and direct precursors of more derived, bi-substituted, long wavelength MAAs (Portwich and Garcia-Pichel 2003) In most cyanobacteria, they are synthesized when exposed to UVB. However, in all but one of the cyanobacteria examined (*Nostoc*), these compounds occur in the cytoplasm and could serve as alternative targets for perhaps only about 10–30% of UVB photons penetrating a cell (Garcia-Pichel and Castenholz 1993, Garcia-Pichel et al. 1993). In cells of small diameter (<10 μm), the beneficial effect would be negligible (Garcia-Pichel 1994). MAAs appear to be important in some way in UVB protection, since they are so tightly associated with UVB exposure. Perhaps, MAAs have an unknown function that is merely associated with UVB exposure (Shick and Dunlap 2002). It is apparent that intracellular MAAs cannot provide complete protection from UVB/A radiation. Some species of cyanobacteria apparently do not synthesize MAAs or scytonemin, and therefore utilize only metabolic repair mechanisms for UVB damage (Quesada and Vincent 1997). However, the employment of migratory escape motility in some may avoid UVR exposure altogether (see behavioral strategies later). In terrestrial *Nostoc* cf. *commune*, however, MAAs are bound to oligosaccharides in the inner external glycan sheath (EPS), with scytonemin in the outer portion (Ehling-Schulz et al. 1997; Ehling-Schulz and Scherer 1999). In this case, then, there is no doubt that MAAs, together with scytonemin, provide a nearly perfect spectral shield against UVB as well as UVA (Fig. 19.8). The UV energy absorbed must be dissipated, which is no problem within the sheath, but intracellular absorption may result in transfer of energy to sensitive molecules. Many cyanobacteria synthesize one or more MAAs even if they have no extracellular sheath or EPS. However, it is probable that most or all sheathed scytonemin producers are capable of making MAAs either constitutively or by UVB induction (Garcia-Pichel and Castenholz 1993). Various MAAs are induced through the absorption of UVB radiation (Ehling-Schulz et al. 1997; Sinha et al. 2001). However, Portwich and Garcia-Pichel (1999) have shown that either UVB or osmotic stress induced MAA synthesis in a strain of *Chlorogloeopsis*. The photoreceptor involved in the induction of MAA synthesis is a pterin with a distinct absorption peak at 310 nm in one cyanobacterial strain (Portwich and Garcia-Pichel 2000). Biosynthetic pathways of MAA synthesis have now been reported (Balskus and Walsh 2010; Gao and Garcia-Pichel 2011).

High levels of MAAs accumulate in cyanobacterial cells of hypersaline environments (Oren 1997), suggesting they could function as compatible solutes in osmoprotection; but this role, while suggested by the author, can only be minor in comparison to that of known compatible solutes in the same cells, the concentration of which may be very much higher.

MAAs and scytonemin are absent in many cyanobacteria (e.g. *Fischerella* [=*Mastigocladus*], *Synechococcus* spp. and many *Leptolyngbya* [=*Phormidium*]) types from hot springs, as well as in vertically migrating types in soft microbial mats, and from many others in the Culture Collection of Microorganisms from Extreme Environments (CCMEE) at the University of Oregon (Castenholz and Garcia-Pichel 2000 and Castenholz, unpublished data) and in the Pasteur Culture Collection. It is these that must have other means of tolerating UVR or avoiding it. In the case of exposure to full solar irradiance, without any possibility of escape because of various features of the habitat, it is apparent that only efficient repair mechanisms can make up for damage done during daylight hours of clear skies with summer insolation at temperate latitudes (Miller et al. 1998). Most repairs probably take place during darkness or during periods of low light as in morning or before dusk. Cyanobacteria are known to have a very efficient daytime photoreactivation system as compared to *Escherichia coli* (Castenholz and Garcia-Pichel 2000). There is, however, little known of UVR effects and tolerance capability outside of the laboratory, with the exception of papers on cyanobacteria in hot springs (Miller et al. 1998; Dillon et al. 2003; Norris et al. 2002). Although some dinoflagellates have the ability to swim vertically out of the damaging intensities of UVB, this is not true of the marine planktonic cyanobacteria that depend on vertical circulation. Gas-vesiculate cyanobacteria in oceanic waters of the tropics and sub-tropics (e.g. *Trichodesmium*) may circulate to great depths, but in very calm conditions may rise to near the surface and become exposed to detrimental UVR.

19.5.2 UVA

UVA may have both direct and indirect effects on cyanobacteria and algae. NAD(P)H (component cofactor in energy metabolism of anoxygenic and oxygenic phototrophs) and other essential nucleotides with absorption maxima at ~340 nm may be regarded as direct targets of UVA. UVA also has some of the same effects as UVB but with less consequence. Pterines can be additional targets or sensitizers. UVA radiation affects many phenomena negatively, but the exact mechanism is not often known. UVA photooxidative damage primarily involves the guanine components of DNA as a result of singlet oxygen generation (Ravanat et al. 2001), which necessitates a photosensitizing compound. Photosynthesis, growth rate and chlorophyll synthesis are negatively affected by UVA. However, the main effects may be through the production of reactive oxygen that may also be produced by the violet/blue wavelengths if the intensity is sufficiently high. The products are singlet oxygen ($^1O_2$), peroxy radicals, and free radical reactions such as the production of OH• (see Asada and Takahashi 1987). Targets of oxidative damage include unsaturated lipids such as phospholipids (Girotti 2001).

UVA (with UVB excluded) resulted in decreases in photosynthetic rate in various cyanobacteria, such as thermophilic *Synechococcus* (Miller et al. 1998), marine *Oscillatoria* and *Spirulina* (Kruschel and Castenholz 1998), Antarctic *Oscillatoria* (Nadeau et al. 1999), and thermophilic *Mastigocladus* (*Fischerella*) (Castenholz, unpublished data) all in outdoor experiments in which the effects of visible irradiance alone, vis+UVA, and vis+UVA and B were compared. The specific targets of these UVA effects have not been investigated.

Photosynthetic prokaryotes may prevent or alleviate the various detrimental effects of UVA (320–400 nm) and short wavelength visible radiation (400–~500 nm) by synthesizing or maintaining a high carotenoid content which may act as an effective antioxidant or "quencher" of reactive oxygen. This may inhibit lipid peroxidation, and thus stabilize membranes (Britton 1995; Niyogi 1999). A high photon flux in excess of what can be used in the photosynthetic reaction may still be absorbed by tetrapyrroles (e.g. chlorophylls and phycobilins) and will result at least in triplet chlorophyll (^{3}Chl) and resultant $^1O_2$ production. Specific carotenoids (e.g. myxoxanthophyll, echinenone, zeaxanthin) may be effective through the thermal dissipation of excitation energy of chlorophyll and singlet oxygen. Recent evidence shows that singlet oxygen may be quenched by carotenoid-cyclodextrin complexes (Kanofsky and Sima 2009). It is probable that a high ratio of effective carotenoids to photosensitizer (i.e. tetrapyrroles) may be extremely important, particularly at suboptimal temperatures when repair and synthetic processes are slow (see Castenholz and Garcia-Pichel 2000). The protective xanthophyll cycle occurs in eukaryotes *in vivo*, but not in prokaryotic phototrophs (Josue and Frank 2002). Zeaxanthin alone, however, may be sufficient for some degree of photoprotection in cyanobacteria (Niyogi 1999). One of the most conspicuous aspects of high light intensity regimes in natural communities of photosynthetic prokaryotes (e.g. hot spring mats) is the yellow or orange colouration in summer in regions of high light intensity with a very high ratio of carotenoids to chlorophyll and phycobilins (Norris et al. 2002; Vincent et al. 1993). However, the effect of carotenoids as a UVR screen is minimal, since almost all of carotenoid absorption is in the violet/blue/green region of the visible spectrum in prokaryotes. Carotenoids, will absorb these wavelengths and be of great benefit directly under high solar intensity. In some cases certain carotenoids may function mainly as major or partial light harvesting complexes, although this is the case mainly in anoxygenic phototrophic bacteria and various algae (e.g. diatoms, chrysomonads, dinoflagellates)(Castenholz and Garcia-Pichel 2000).

Superoxide radical anions are also produced with high light intensity. For example, increased synthesis of Mn SOD above constitutive levels of Fe SOD may occur in cyanobacteria (Castenholz and Garcia-Pichel 2000). H_2O_2, a product of SOD activity, is considered to be relatively harmless compared to superoxide. In *Synechocystis* PCC 6803, peroxide decomposition is catalyzed by catalase-peroxidase and a thiol-specific peroxidase (Tichy and Vermaas 1999). Tocopherols, ascorbates, and glutathione are also used as peroxide detoxifying compounds (Niyogi 1999).

An important preventative and passive method of protection from UVA results from the presence of scytonemin in the sheath or EPS of cyanobacteria, but this does not apply to all cyanobacteria. Only those that possess some type of EPS and are commonly exposed to UVR (even low intensity UVR) synthesize scytonemin. This includes numerous surface layers of microbial mats in flats infrequently covered by tidal waters (Cockell and Rothschild 1999; Fleming et al. 2007), lower temperature (<~50°C) hot spring mats (Brenowitz and Castenholz 1997; Dillon et al. 2003), terrestrial mats and crusts (Garcia-Pichel and Belnap 1996), shallow and clear oligotrophic fresh waters (Johnson and Castenholz 2000), cyanolichens (Büdel et al. 1997) and many other exposed habitats (Garcia-Pichel and Castenholz 1991; Castenholz and Garcia-Pichel 2000). Scytonemin, as mentioned earlier, has major absorption peaks at ~250 and 370–372 nm (*in vivo*). It is a unique dimeric indole alkaloid with a molecular weight of 544 (Proteau et al. 1993). In mature surface filaments of cyanobacteria scytonemin-rich sheaths may screen out >95% of the UVA and a substantial portion of UVB (see above). This passive method of preventing or slowing the inhibition caused by UVA radiation apparently results in great benefits in terms of fitness and survival, particularly in habitats where desiccation or suboptimal growth conditions prevail (Garcia-Pichel et al. 1992;

Brenowitz and Castenholz 1997; Dillon et al. 2003; Dillon and Castenholz 2003).

The synthesis of scytonemin is initiated and sustained by exposure to UVA although violet/blue wavelengths have some effect (Garcia-Pichel and Castenholz 1991), but some rock-inhabiting cyanobacteria synthesize a small content of scytonemin constitutively (Castenholz, unpublished data). Stresses such as increased temperature and photooxidative conditions, in conjunction with UVA, caused a small increase in scytonemin production in one species of *Chroococcidiopsis* (Dillon et al. 2002). In addition, a moderate osmotic stress, without UVR or blue light, resulted in some scytonemin synthesis. Periodic desiccation in addition to UVA exposure also increased scytonemin content (Fleming and Castenholz 2007). It has been shown that the content of scytonemin per unit area in intertidal *Lyngbya* cf. *aestuarii* increased during the higher solar irradiance period of summer (Karsten et al. 1998). Another stress that promoted the synthesis of scytonemin is diazotrophic growth, a condition that could be considered a stress because of the high energy requirement for N_2-fixation (Fleming and Castenholz 2008). In this case, *Nostoc* ATCC 29133 produced scytonemin under UVA radiation only when no combined nitrogen was available.

The genetic analyses of a scytonemin-less *Nostoc punctiforme* ATCC 29133 mutant (Soule et al. 2007), have opened the door in recent years to the study of the molecular basis of scytonemin biology. Much has been accomplished in this respect in a short time. The mutant implicated a genomic region containing 18 contiguous genes in the synthesis of scytonemin. Similar regions have been since found by comparative genomics or by direct sequencing in a variety of other cyanobacteria (Sorrels et al. 2009; Soule et al. 2009a). Homologous regions in non-heterocystous strains (*Lyngbya* and *Cyanothece*) contain 5 additional genes in the cluster (Soule et al. 2009a). These are also conserved in *Nostoc* and other heterocystous cyanobacteria, where they are non-contiguous. Most of the genes in the upstream region of the *Nostoc* cluster, with the exception of *scyA* and *scyB* that code for novel proteins without an easily predictable function, but a transposon inserted in *scyD* effectively eliminated scytonemin biosynthesis under otherwise inducing conditions. Balskus and Walsh (2008) could subsequently show that the protein coded for in one of these genes (*scy*A) catalyzed the condensation of phenol- and indole-pyruvate moities, a fact that is in agreement with the expected scytonemin synthetic pathway, and with the pyruvate-condensing activity predicted for s*cy*A on the basis of sequence similarity. The functions of most of the other upstream genes have not been clarified, but tentative assignments on the basis of comparative genomics have been carried out for some (Soule et al. 2009a). Downstream in the cluster, one finds redundant orthologs of genes coding for enzymes in the aromatic amino acid pathway. Those present suffice to lead to *p*-hydroxyphenyl-pyruvate from end products of the shikimic acid pathway. Redundant copies of the genes coding for the key regulatory and rate-limiting enzymes of the shikimic acid pathway are found downstream as well. Thus, it seems that the downstream region is dedicated to the delivery of monomeric blocks for scytonemin synthesis. Additionally, two genes immediately upstream from the scytonemin-associated cluster likely encode a two-component sensor kinase and response regulator, respectively. These also are highly conserved in sequence and location among cyanobacterial strains that possess the scytonemin cluster, and may be involved in regulating the expression of the scytonemin-related genes. As expected the expression of all genes in the cluster is positively regulated by exposure to UVA, and transcription proceeds in an operon-like fashion as a single transcript (Soule et al. 2009b). In recent years, much has been learned about the biosynthesis of scytonemin, although it is uncertain in what form it is transported or assembled in the sheath or EPS. Since *scy*D, *scy*E, and *scy*F, have export signal domains, and others have putative transmembrane domains, it can be inferred that scytonemin biosynthesis is compartmentalized within the cell, and that monomer synthesis and initial condensation are cytoplasmic. Later reactions are predicted to be periplasmic (Soule et al. 2009a) (Fig. 19.9).

As an aside, it appears that an unknown compound is present in many marine and freshwater green macro- and micro-algae that acts an UVA and UVB shield with a relatively high protective efficiency (Pescheck et al. 2010). In the case of green freshwater micro-algae there appear to be macromolecules (possibly sporopollenin-like compounds, i.e. algenans) bound to the cell walls.

19.6 Behavioural Methods of Escaping UVR Exposure

Although much is known about vertical movements and regulation of buoyant or semi-buoyant gas-vacuolate cyanobacteria, and anoxygenic phototrophic bacteria in the water column of lakes (Walsby 1994), work that focuses on the effects of the UV portion of the spectrum is lacking.

Gliding filamentous cyanobacteria (as well as a few species of unicellular forms) often move vertically in soft microbial mats and sediments in response to high intensity visible light and UVR (for a summary, see Castenholz and Garcia-Pichel 2000). The downward movement of filamentous cyanobacteria (0.4–>1 mm) in dense microbial mats has been shown to be a response to high solar irradiance, particularly UVR, both UVB and UVA in temperate habitats, hot springs, and cold Antarctic ponds (Garcia-Pichel et al. 1994; Ramsing and Prufert-Bebout 1994; Bebout and Garcia-Pichel 1995; Kruschel and Castenholz 1998; Richardson and Castenholz 1987; Nadeau et al. 1999) (Fig. 19.10). In general, the return towards or to the mat surface occurred in darkness or in dim daylight until UVR

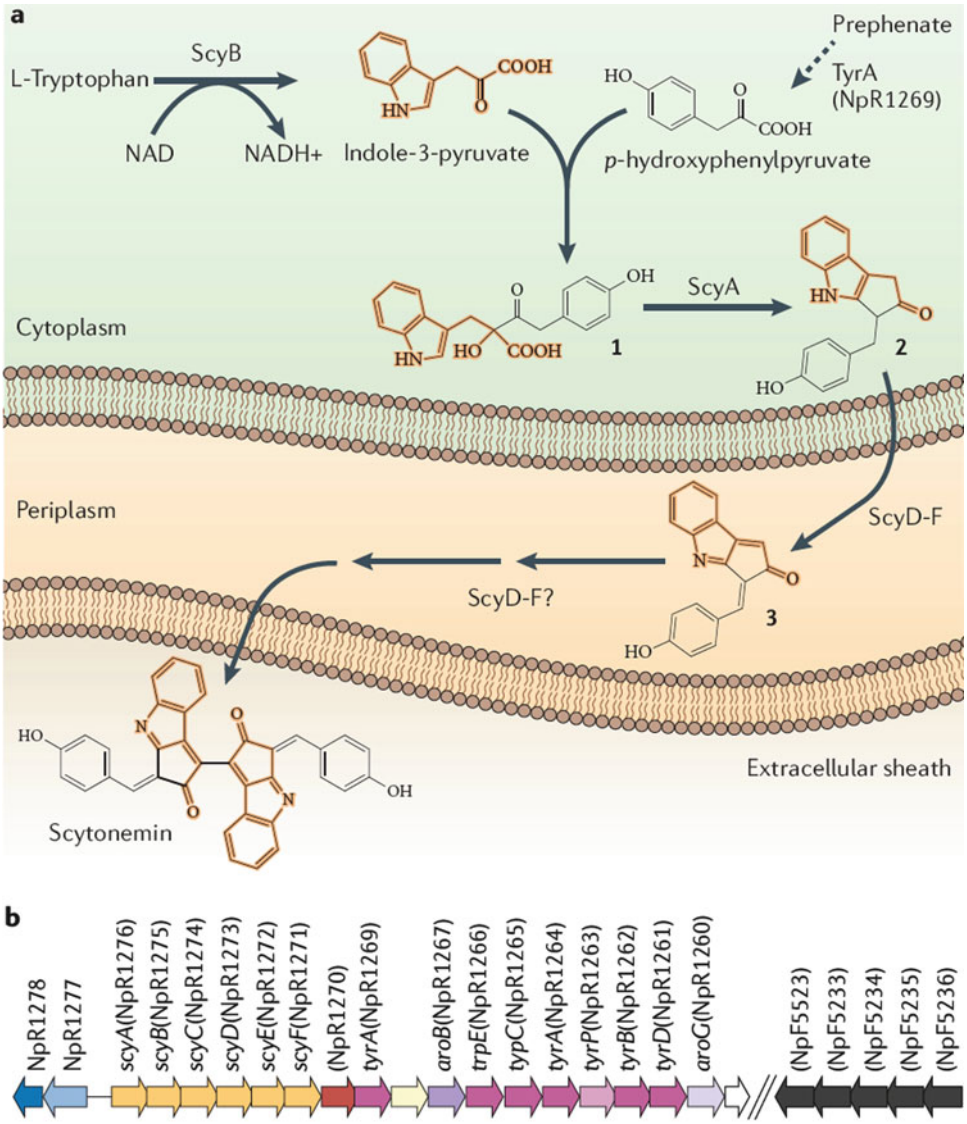

Fig. 19.9 Working model of scytonemin biosynthesis in *Nostoc punctiforme* based on genomic and enzymological analyses: UVA is absorbed and activates the genes in the scytonemin operon (**b**), the gene products of which catalyze the sequence of reactions depicted in (**a**), likely in a compartimentalized fashion. The genes in purple color constitute redundant orthologs of key shikimic acid and aromatic amino acid biosynthetic pathways. (From Gao and Garcia-Pichel 2011, Fig. 3, p 795. Courtesy of Nature Publishing Company)

or high visible light intensities again appear to stop the upward movement (Garcia-Pichel et al. 1994; Kruschel and Castenholz 1998). In two cases it has been shown that failure to migrate downward from the surface during periods of high solar irradiance (including UVR) would have resulted in extreme inhibition of photosynthesis or death (Garcia-Pichel et al. 1994; Kruschel and Castenholz 1998). Filamentous and unicellular cyanobacteria have also been shown to move vertically in soft hot spring mats, also as a response to high light intensity, but UVR was not specifically taken into account (Castenholz 1968; Richardson and Castenholz 1987; Ramsing et al. 1997). In almost all of the cases cited, the cyanobacteria involved did not possess MAAs nor did they have the ability to withstand large doses of UVR. Thus, escape was essentially the only tactic for survival. Garcia-Pichel et al. (1994) have also shown in one case, by the use of micro light sensors, that the gradual descent of *Spirulina* and *Oscillatoria* to ~0.5 mm and later the ascent in a soft hypersaline mat would result in maximum photosynthetic rates during most daylight hours. Thus, the daytime descent may not be simply an escape but a finely regulated optimization of light available for photosynthesis. These cyanobacteria also retained high cellular contents of chlorophyll *a* and phycocyanin. Retaining this condition would be distinctly advantageous during overcast periods of low light intensity and during early morning and late afternoon when these cyanobacteria would be at the mat surface and able to absorb the low photon flux more efficiently for

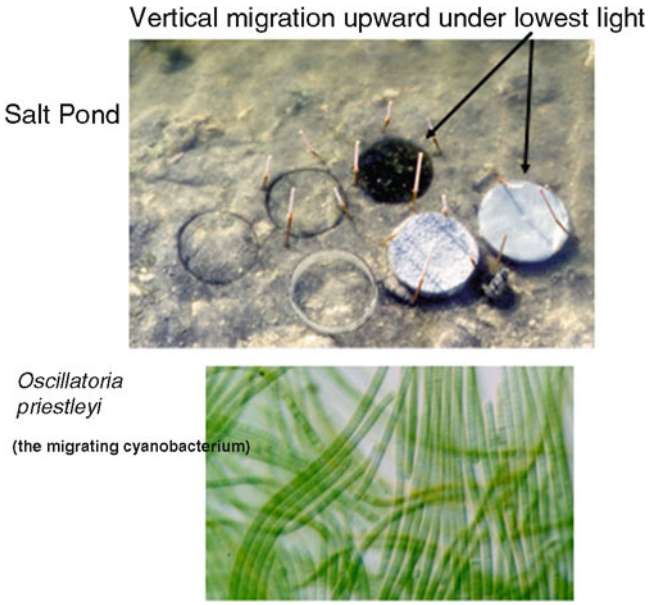

Fig. 19.10 Upward migration of *Oscillatoria* cf. *priestleyi* in hypersaline "Salt Pond" (Bratina Island area, Antarctica), under the lowest light intensity with filter removed after 4 h of treatment (*upper right dark area*). The intensity was ~5–8 Wm^{-2} of PAR with no detectable UVR, and the temperature 6–8°C. The same result occurred under darkness, but not under ~12–20 Wm^{-2} (*next filter to left*) where UVA was barely measurable, or under full solar radiation (230–360 Wm^{-2}) last ring to *left*. A more intricate series of experiments (also in "Salt Pond") are described by Nadeau et al. (1999). The rate of movement was about 50% slower than that observed for motile cyanobacteria in temperate mats in Baja California (Kruschel and Castenholz 1998). *Oscillatoria* cf. *priestleyi* (~7 μm trichome diameter) is shown in the lower panel (Photos by RWC)

photosynthetic activity. A high content of chlorophyll and/or phycocyanin could also become a detriment and act as photosensitizers if the cells were suddenly exposed to high solar irradiance. In more sedentary cyanobacteria the synthesis of these pigments are regulated downward by stopping synthesis or by regulated degradation, thus increasing the ratio of carotenoids to photosensitizers, allowing an increased tolerance to photo-oxidative stress.

19.7 Effects of Decrease of UVR on Phototrophic Communities

Bothwell et al. (1993, 1994) studied the effects of 3–5 weeks of UVR applied to a lotic community that colonized an artificial substrate. As a result of enhanced UVB a greater biomass of diatoms accumulated than in the control. However, chironomid larvae that normally graze on diatoms were more sensitive than the diatoms to UVB. Since these were indoor experiments the +UV treatment may be closer to natural field conditions than those of the controls. Epiphytic cyanobacterial mats from tropical mangrove communities were subjected to +UV and -UV treatments for 27 days with laboratory

Table 19.1 Multiple methods of tolerating UV radiation by cyanobacteria (From Castenholz 2004, Table 1, p 457. With kind permission from Springer Science + Business Media B.V.)

Method	A	B	C
Scytonemin	Yes	No	No
MAAs	Most yes	Many yes	Many yes
Motility-avoidance	No	Yes	No
Chlorophyll-phycobilin-carotenoid regulation	Yes	Yes	Yes
Carotenoid quenching	Yes	Yes	Yes
SOD, peroxidase, etc.	Yes	Yes	Yes
Repair – synthesis	Yes	Yes	Yes

A sheathed types, B motile types (filamentous & unicellular), C no motility, sheath or EPS. Motile hormogonial stages of type A may avoid UVR

UVB+UVA+PAR, UVA+PAR, or PAR only. Large differences in species arrangement and abundance occurred (Sheridan 2001). With UVA and UVB the top layer of scytonemin-containing, diazotrophic *Nostoc* cf. *commune* and *Scytonema* sp. were maintained as in the natural mat. When UVR was excluded, the *Nostoc* was overrun or overgrown by a less UV-tolerant species of *Phormidium* which was not capable of diazotrophy. As a result, overall N_2-fixation decreased greatly.

Two alkaline geothermal streams in Yellowstone National Park (40–47°C) were covered for 1–3 months with filters that excluded or transmitted UVR (Norris et al. 2002). There were no apparent cyanobacterial community composition changes during the summer with or without high or low UVR, as assessed by DGGE (denaturing gradient gel electrophoresis). Although the cyanobacterial composition of these communities was apparently stable, surface layers of cyanobacteria protected by filters from UV radiation were not as competent photosynthetically as those that had been maintained under UVR. This decrease in competence was expressed as a temporary loss of the ability to perform at a maximum rate under full solar irradiance (including UVR). Recovery occurred within a week under full irradiance.

19.8 Conclusions

UV radiation effects on microbial populations have been studied in recent years with increasing frequency and with refined molecular methodology. One reason is the awareness of decreasing levels of ozone in the stratosphere in some regions that result in increased UVB flux. Microbial populations may exhibit a more immediate and measurable sensitivity to small increases in UVR than larger macrophytes and metazoans that may require weeks, months, or years to show UVR effects. Some cyanobacteria, representing descendants of the oldest oxygenic inhabitants of the planet, have evolved many methods (or strategies) for coping with present levels of UVR (Table 19.1).

Since cyanobacteria have invaded (or remain as relics) in a large number of extreme environments, including shallow water and terrestrial surfaces, they currently must often cope with high solar irradiance in which UVR can be the most detrimental factor. They have done so by evolving sunscreen pigments that envelope the cells and work even when cells are at rest, and by synthesizing other compounds such as mycosporine-like amino acids (the true value of which is still not completely evaluated). Also, they have probably evolved several efficient methods of dissipating excess photon energy, and by using regulated systems for repair of damaged DNA and for replacement of UV damaged compounds, and by using directed, "phototactic" gliding motility in soft microbial mats or sediments for escaping the daytime high intensities of solar irradiance.

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References

Asada K, Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CB, Arntzen CJ (eds) Photoinhibition. Elsevier Science Publishers, Amsterdam, pp 227–287

Bailey S, Grossman A (2008) Photoprotection in cyanobacteria: regulation of light harvesting. Photochem Photobiol 84:1410–1420

Balskus EP, Walsh CT (2008) Investigating the initial steps in the biosynthesis of cyanobacterial sunscreen scytonemin. J Am Chem Soc 130:15260–15261

Balskus EP, Walsh CT (2010) The genetic and molecular basis of sunscreen biosynthesis in cyanobacteria. Science 329:1653–1656

Bebout BM, Garcia-Pichel F (1995) UVB-induced vertical migrations of cyanobacteria in a microbial mat. Appl Environ Microbiol 61:4215–4222

Billi D, Friedmann EI, Hofer KG, Caiola MG, Ocampo-Friedmann R (2000) Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. Appl Environ Microbiol 66:1489–1492

Bonilla SE, Villeneuve V, Vincent WF (2005) Benthic and planktonic algal communities in a High Arctic lake: pigment structure and contrasting responses to nutrient enrichment. J Phycol 41:1120–1130

Booth CR, Morrow JH (1997) The penetration of UV into natural waters. Photochem Photobiol 65:254–257

Bothwell ML, Sherbot D, Roberg AC, Daley RJ (1993) Influence of natural ultraviolet radiation on lotic periphytic diatom community growth, biomass accrual, and species composition: short-term versus long-term effects. J Phycol 29:24–35

Bothwell ML, Sherbot DMJ, Pollock CM (1994) Ecosystem response to solar ultraviolet-B radiation: influence of trophic level interactions. Science 265:97–100

Bouchard JN, Roy S, Ferreyra G, Campbell DA, Curtosi A (2005) Ultraviolet-B effects on photosystem II efficiency of natural phytoplankton communities from Antarctica. Polar Biol 28:607–618

Brenowitz S, Castenholz RW (1997) Long-term effects of UV and visible irradiance on natural populations of a scytonemin-containing cyanobacterium (*Calothrix* sp.). FEMS Microbiol Ecol 24:343–352

Britton G (1995) Structure and properties of carotenoids in relation to function. FASEB J 9:1551–1558

Büdel B, Karsten U, Garcia-Pichel F (1997) Ultraviolet-absorbing scytonemin and mycosporine-like amino acid derivatives in exposed, rock-inhabiting cyanobacterial lichens. Oecologia 112:165–172

Campbell D, Eriksson M-J, Öquist G, Gustafsson P, Clarke AK (1998) The cyanobacterium *Synechococcus* resists UV-B by exchanging photosystem II reaction center D1 proteins. Proc Natl Acad Sci USA 95:364–369

Castenholz RW (1968) The behavior of *Oscillatoria terebriformis* in hot springs. J Phycol 4:132–139

Castenholz RW (2004) Phototrophic bacteria under UV stress. In: Seckbach J (ed) Origins, evolution and biodiversity of microbial life. Kluwer Academic Publishers, Dordrecht, pp 445–461

Castenholz RW (2009) Mats, microbial. In: Encyclopedia of Microbiology, Elsevier, pp 278–292

Castenholz RW, Garcia-Pichel F (2000) Cyanobacterial responses to UV-radiation. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 591–611, 669 pp

Castenholz RW, Schneider A (1993) Cyanobacterial dominance at high and low temperatures: optimal conditions or precarious existence? In: Guerrero R, Pedros-Alio C (eds) Trends in microbial ecology. Spanish Society for Microbiology, Barcelona, pp 19–24

Choi J-S, Chung Y-H, Moon Y-J, Kim C, Watanabe M, Song P-S, Joe C-O, Bogorad L, Park YM (1999) Photomovement of the gliding cyanobacterium *Synechocystis* sp. PCC 6803. Photochem Photobiol 70:95–102

Cockell CS (1998) The biological effects of high ultraviolet radiation on early Earth: a theoretical evaluation. J Theor Biol 193:717–729

Cockell CS (2001) A photobiological history of Earth. In: Cockell CS, Blaustein AR (eds) Ecosystems, evolution, and ultraviolet radiation. Springer, New York, pp 1–35, 221 pp

Cockell CS, Rothschild LJ (1999) The effects of UV radiation A and B on diurnal variation in photosynthesis in three taxonomically and ecologically diverse microbial mats. Photochem Photobiol 69:203–210

Cullen JJ, Neale PJ (1994) Ultraviolet radiation, ozone depletion and marine photosynthesis. Photosynth Res 39:303–320

Day TA, Neale PJ (2002) Effects of UV-B radiation on terrestrial and aquatic primary producers. Annu Rev Ecol Syst 33:371–396

Des Marais DJ (1995) The biogeochemistry of hypersaline microbial mats. Adv Microbiol Ecol 14:251–274

Diffey BL, Green AT, Loftus MJ, Johnson GJ, Lee PS (1995) A portable instrument for measuring ground reflectance in the ultraviolet. Photochem Photobiol 61:68–70

Dillon JG, Castenholz RW (1999) Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life. J Phycol 35:673–681

Dillon JG, Castenholz RW (2003) The synthesis of the UV-screening pigment, scytonemin, and photosynthetic performance in isolates from closely related natural populations of cyanobacteria (*Calothrix* sp.). Environ Microbiol 5:484–491

Dillon JG, Tatsumi CM, Tandingan PG, Castenholz RW (2002) Effect of environmental factors on the synthesis of scytonemin, a UV-screening pigment, in a cyanobacterium (*Chroococcidiopsis* sp.). Arch Microbiol 177:322–331

Dillon JG, Miller SR, Castenholz RW (2003) UV-acclimation responses in natural populations of cyanobacteria (*Calothrix* sp.). Environ Microbiol 5:473–483

Dodds WK, Castenholz RW (1988) The biological effects of nitrate fertilization and water replacement in an oligotrophic cold water pond. Hydrobiologia 162:141–146

Eguchi M, Oketa T, Miyamoto N, Maeda H, Kawai A (1996) Occurrence of viable photoautotrophic picoplankton in the aphotic zone of Lake Biwa, Japan. J Plankton Res 18:539–550

Ehling-Schulz M, Scherer S (1999) UV protection in cyanobacteria. Eur J Phycol 34:329–338

Ehling-Schulz M, Bilger W, Scherer S (1997) UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. J Bacteriol 179:1940–1945

Fleming ED, Castenholz RW (2007) Effects of periodic desiccation on the synthesis of the UV-screening compound, scytonemin, in cyanobacteria. Environ Microbiol 9:1448–1455
Fleming ED, Castenholz RW (2008) Effects of nitrogen source on the synthesis of the UV-screening compound, scytonemin, in the cyanobacterium *Nostoc punctiforme* PCC 73102. FEMS Microbiol Ecol 63:301–308
Fleming ED, Bebout BM, Castenholz RW (2007) Effects of salinity and light intensity on the resumption of photosynthesis in a rehydrated cyanobacterial mat from Baja California Sur, Mexico. J Phycol 43:15–24
Frederick JE, Snell HE, Haywood EK (1989) Solar ultraviolet radiation at the earth's surface. Photochem Photobiol 50:443–450
Gao K, Ye C (2007) Photosynthetic insensitivity of the terrestrial cyanobacterium *Nostoc flagelliforme* to solar UV radiation while rehydrated or desiccated. J Phycol 43:628–635
Gao K, Li P, Watanabe T, Heibling EW (2008) Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of *Arthrospira (Spirulina) platensis* (Cyanophyta). J Phycol 44:777–786
Gao Q, Garcia-Pichel F (2011) Microbial ultraviolet sunscreens. Nat Rev Microbiol 9:791–802
Garcia-Pichel F (1994) A model for internal self-shading in planktonic microorganisms and its implications for the usefulness of sunscreens. Limnol Oceanogr 39:1704–1717
Garcia-Pichel F (1998) Solar ultraviolet and the evolutionary history of cyanobacteria. Orig Life Evol Biosph 28:321–347
Garcia-Pichel F, Bebout BM (1996) The penetration of ultraviolet radiation into shallow water sediments: high exposure for photosynthetic communities. Mar Ecol Prog Ser 131:257–262
Garcia-Pichel F, Belnap J (1996) Microenvironments and microscale productivity of cyanobacterial desert crusts. J Phycol 32:774–782
Garcia-Pichel F, Castenholz RW (1991) Charaterization and biological implications of scytonemin, a cyanobacterial sheath pigment. J Phycol 27:395–409
Garcia-Pichel F, Castenholz RW (1993) Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl Environ Microbiol 59:163–169
Garcia-Pichel F, Sherry ND, Castenholz RW (1992) Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlrogloeopsis* sp. Photochem Photobiol 56:17–23
Garcia-Pichel F, Wingard CE, Castenholz RW (1993) Evidence regarding the UV-sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. Appl Environ Microbiol 59:170–176
Garcia-Pichel F, Mechling M, Castenholz RW (1994) Diel migrations of microorganisms within a benthic, hypersaline mat community. Appl Environ Microbiol 60:1500–1511
Gieskes WWC, Buma AGJ (1997) UV damage to plant life in a photobiologically dynamic environment: the case of marine phytoplankton. Plant Ecol 128:16–25
Gill RT, Katsoulakis E, Schmitt W, Taroncher-Oldenburg G, Misra J, Stephanopoulos G (2002) Genome-wide dynamic transcriptional profiling of the light-to-dark transition in *Synechocystis* sp. strain PCC 6803. J Bacteriol 184:3671–3681
Girotti AW (2001) Photooxidized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. J Photochem Photobiol B 63:103–113
Häder D-P (1984) Effects of UV-B on motility and photoorientation in the cyanobacterium, *Phormidium uncinatum*. Arch Microbiol 140:34–39
Häder D-P (2001) Ultraviolet radiation and aquatic microbial ecosystems. In: Cockell CS, Blaustein AR (eds) Ecosystems, evolution, and ultraviolet radiation. Springer, New York, pp 150–169
Häder D-P, Lebert M, Schuster M, Del Ciampo L, Helbling EW, McKenzie R (2007) ELDONET-a decade of monitoring solar radiation on five continents. Photochem Photobiol 83:1348–1357
He Y-Y, Klisch M, Häder D-P (2002) Adaptation of cyanobacteria to UV-B stress correlated with oxidative stress and oxidative damage. Photochem Photobiol 76:188–196
Helbling EW, Villafañe V, Holm-Hansen O (1994) Effects of ultraviolet radiation on Antarctic marine plankton photosynthesis with particular attention to the influence of mixing. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects, vol 62. American Geophysical Union, Washington, DC, pp 207–227
Hockberger PE (2002) A history of ultraviolet photobiology for humans, animals and microorganisms. Photochem Photobiol 76:561–579
Holm-Hansen O, Lubin D, Helbling EW (1993) Ultraviolet radiation and its effects on organisms in aquatic environments. In: Young AR, Björn LO, Moan J, Nultsch W (eds) Environmental UV photobiology. Plenum Press, New York, pp 379–425
Hren MT, Tice MM, Chamberlain CP (2009) Oxygen and hydrogen isotope evidence for a temperate climate 3.42 billion years ago. Nature 462:205–208
Jägger J (1985) Solar-UV action on living cells. Praeger, New York
Javor BJ, Castenholz RW (1981) Laminated microbial mats, Laguna Guerrero Negro, Mexico. Geomicrobiology 2:237–273
Javor BJ, Castenholz RW (1984) Productivity studies of microbial mats, Laguna Guerrero Negro, Mexico. In: Cohen Y, Castenholz RW, Halvorson HO (eds) Microbial mats: stromatolites. Alan R. Liss, Inc., New York, pp 149–170
Jeffrey WH, Pledger RJ, Aas P, Hager S, Coffin RB, Von Haven R, Mitchell DL (1996) Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation. Mar Ecol Prog Ser 137:283–291
Johnson AC, Castenholz RW (2000) Preliminary observations of the benthic cyanobacteria of Waldo Lake and their potential contribution to lake productivity. Lake Reserv Manag 16:85–90
Josue JS, Frank HA (2002) Direct determination of the S_1 excited-state energies of xanthophylls by low-temperature fluorescence spectroscopy. J Phys Chem A 106:2815–2824
Kanofsky JR, Sima PD (2009) Quenching of singlet oxygen by a carotenoid-cyclodextrin complex: the importance of aggregate formation. Photochem Photobiol 85:391–399
Karlsson J, Byström P, Ask J, Ask P, Persson L, Jansson M (2009) Light limitation of nutrientpoor lake ecosystems. Nature 460:506–509
Karsten U, Maier J, Garcia-Pichel F (1998) Seasonality in UV-absorbing compounds of cyanobacterial mat communities from an intertidal mangrove. Aquat Microb Ecol 16:37–44
Kasting JF (1987) Theoretical constraints on oxygen and carbon dioxide concentrations in the Precambrian atmosphere. Precambrian Res 34:205–229
Kasting JF, Zahnle KJ, Pinto JP, Young AT (1989) Sulfur, ultraviolet radiation, and the early evolution of life. Orig Life Evol Biosph 19:95–108
Kelman D, Ben-Amotz A, Berman-Frank I (2009) Carotenoids provide the major antioxidant defence in the globally significant N_2-fixing marine cyanobacterium *Trichodesmium*. Environ Microbiol 11:1897–1908
Knoll AH (2008) Cyanobacteria and earth history. In: Herrero A, Flores E (eds) The cyanobacteria – molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 1–19, 484 pp
Koller LR (1965) Ultraviolet radiation. Wiley, New York, 312 pp
Kruschel C, Castenholz RW (1998) The effect of solar UV and visible irradiance on the vertical movements of cyanobacteria in microbial mats of hypersaline waters. FEMS Microbiol Ecol 27:53–72
Kvalevåg MM, Myhre G, Lund Myhre CE (2009) Extensive reduction of surface UV radiation since 1750 in world's populated regions. Atmos Chem Phys 9:7737–7751
Lao K, Glazer AN (1996) Ultraviolet-B photodestruction of a light-harvesting complex. Proc Natl Acad Sci USA 93:5258–5263
Laurion I, Roy S (2009) Growth and photoprotection in three dinoflagellates (including two strains of *Alexandrium tamarense*)

and one diatom exposed to four weeks of natural and enhanced ultraviolet-B radiation. J Phycol 45:16–33

Lehr CR, Frank SD, D'Imperio S, Kalinin A, Toplin JA, Norris TB, Castenholz RW, McDermott TR (2007) Cyanidial (Cyanidiales) population diversity and dynamics in an acid-sulfate chloride spring in Yellowstone National Park. J Phycol 43:3–14

Los DA, Suzuki I, Zinchenko VV, Murata N (2008) Stress responses in *Synechocystis*: regulated genes and regulated systems. In: Herrero A, Flores E (eds) The cyanobacteria – molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 117–157, 484 pp

MacDonald TM, Dubois L, Smith LC, Campbell DA (2003) Sensitivity of cyanobacterial antenna, reaction center and CO_2 assimilation transcripts and proteins to moderate UVB: light acclimation potentiates resistance to UVB. Photochem Photobiol 77:405–412

Máté Z, Sass L, Szekeres M, Vass I, Nagy F (1998) UV-B-induced differential transcription of *psbA* genes encoding the D1 protein of photosystem II in the cyanobacterium *Synechocystis* 6803. J Biol Chem 273:17439–17444

Matthes U, Turner SJ, Larson DW (2001) Light attenuation by limestone rock and its constraint on the depth distribution of endolithic algae and cyanobacteria. Int J Plant Sci 162:262–270

Mazor G, Kidron GJ, Vonshak A, Abeliovich A (1996) The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts. FEMS Microbiol Ecol 21:21–130

Meador JA, Baldwin AJ, Catala P, Jeffrey WH, Joux F, Moss JA, Pakulski D, Stevens R, Mitchell DL (2009) Sunlight-induced DNA damage in marine micro-organisms collected along a latitudinal gradient from 70°N to 68°S. Photochem Photobiol 85:412–420

Miller SR, Wingard CE, Castenholz RW (1998) Effects of visible light and UV radiation on photosynthesis in a population of a hot spring cyanobacterium, a *Synechococcus* sp., subjected to high-temperature stress. Appl Environ Microbiol 64:3893–3899

Morel A, Gentili B, Claustrre H, Babin M, Bricaud A, Ras J, Tièche F (2007) Optical properties of the "clearest" natural waters. Limnol Oceanogr 52:217–229

Nadeau T-L, Castenholz RW (2000) Characterization of psychrophilic oscillatorians (Cyanobacteria) from Antarctic meltwater ponds. J Phycol 36:914–923

Nadeau T-L, Howard-Williams H, Castenholz RW (1999) Effects of solar UV and visible irradiance on photosynthesis and vertical migration of *Oscillatoria* sp (cyanobacteria) in an Antarctic microbial mat. Aquat MicrobEcol 20:231–243

Nicholson P, Osborn RW, Howe CJ (1987) Induction of protein synthesis in response to ultraviolet light, nalidixic acid and heat shock in the cyanobacterium *Phormidium laminosum*. FEBS Lett 221:110–114

Nielsen T, Ekelund NGA (1995) Influence of solar ultraviolet radiation on photosynthesis and motility of marine phytoplankton. FEMS Microbiol Ecol 18:281–288

Nienow JA, Friedmann EI (1993) Terrestrial lithophytic (rock) communities. In: Friedmann EI (ed) Antarctic microbiology. Wiley-Liss, New York, pp 343–412

Niyogi KK (1999) Photoprotection revisited: genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol 50:333–359

Norris TB, Castenholz RW (2006) Endolithic photosynthetic communities within ancient and recent travertine deposits in Yellowstone National Park. FEMS Microbiol Ecol 57:470–483

Norris TB, McDermott TR, Castenholz RW (2002) The long-term effects of UV exclusion on the microbial composition and photosynthetic competence of bacteria in hot-spring microbial mats. FEMS Microbiol Ecol 39:193–209

Olsson-Francis K, de la Torre R, Cockell CS et al (2010) Isolation of novel extreme-tolerant cyanobacteria from a rock-dwelling microbial community by using exposure to low Earth orbit. Appl Environ Microbiol 76:2115–2121

Orce VL, Helbling EW (1997) Latitudinal UVR-PAR measurements in Argentina: extent of the "ozone hole". Glob Planet Change 15:113–121

Oren A (1997) Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. Geomicrobiol J 14: 231–240

Pakker H, Martins RST, Boelen P, Buma AGJ, Nikaido O, Breeman AM (2000) Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in *Palmaria palmata* (Rhodophyta). J Phycol 36:334–341

Pavlov AA, Kasting JF (2002) Mass-independent fractionation of sulfur isotopes in Archean sediments: strong evidence for an anoxic Archean atmosphere. Astrobiology 2:27–41

Pescheck F, Bischof K, Bilger W (2010) Screening of ultraviolet-A and ultraviolet-B radiation in marine green macroalgae (Chlorophyta). J Phycol 46:444–455

Phoenix VR, Bennett PC, Engel AS, Tyler SW, Ferris FG (2006) Chilean high-altitude hot spring sinters: a model system for UV screening mechanisms by early Precambrian cyanobacteria. Geobiology 4:15–28

Pierson BK (1994) The emergence, diversification, and role of photosynthetic eubacteria. In: Bengtson S, Bergström J, Vidal G, Knoll A (eds) Early life on earth. Columbia University Press, New York, pp 161–180, 605 pp

Pierson BK, Mitchell HK, Ruff-Roberts AL (1993) *Chloroflexus aurantiacus* and ultraviolet radiation: implications for Archean shallow-water stromatolites. Orig Life Evol Biosph 23:243–260

Porankiewicz J, Schelin J, Clarke AK (1998) The ATP-dependent Clp protease is essential for acclimation to UV-B and low temperature in the cyanobacterium *Synechococcus*. Mol Microbiol 29:275–283

Portwich A, Garcia-Pichel F (1999) Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogloeopsis* PCC 6912. Arch Microbiol 172:187–192

Portwich A, Garcia-Pichel F (2000) A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912. Photochem Photobiol 71:493–498

Portwich A, Garcia-Pichel F (2003) Biosynthetic pathway of mycosporines (mycosporine-like amino acids) in the cyanobacterium *Chlorogloeopsis* sp. strain PCC 6912. Phycologia 42:384–392

Postius C, Ernst A, Kenter U, Böger P (1996) Persistence and genetic diversity among strains of the phycoerythrin-rich cyanobacteria from the picoplankton of Lake Constance. J Plankton Res 18: 1159–1166

Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755–805

Prezelin BB, Boucher NP, Smith RC (1994) Marine primary production under the influence of the Antarctic ozone hole: Icecolors'90. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurement and biological effects, vol 62. American Geophysical Union, Washington, DC, pp 159–186

Proteau PJ, Gerwick WH, Garcia-Pichel F, Castenholz RW (1993) The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. Experientia 49:825–829

Quesada A, Vincent WF (1997) Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. Eur J Phycol 32:335–342

Ramsing NB, Prufert-Bebout L et al (1994) Motility of *Microcoleus chthonoplastes* subjected to different light intensities quantified by digital image analysis. In: Stal LJ, Caumette P (eds) Microbial mats. Structure, development and environmental significance, vol 35, NATO ASI series. Springer, Berlin, pp 183–191

Ramsing NB, Ferris MJ, Ward DM (1997) Light-induced motility of thermophilic *Synechococcus* isolates from Octopus Spring, Yellowstone National Park. Appl Environ Microbiol 63:2347–2354

Rasmussen B, Blake TM, Fletcher IR, Kilburn MR (2009) Evidence for microbial life in synsedimentary cavities from 2.75 Ga terrestrial environments. Geology 37:423–426

Ravanat J-L, Douki T, Cadet J (2001) Direct and indirect effects of UV radiation on DNA and its components. J Photochem Photobiol B 63:88–102

Richardson LL, Castenholz RW (1987) Diel vertical movements of the cyanobacterium *Oscillatoria terebriformis* in a sulfide-rich hot spring microbial mat. Appl Environ Microbiol 53:2142–2150

Robinson N (1966) Solar radiation. Elsevier, Amsterdam

Roy S (2000) Strategies for the minimization of UV-induced damage. In: de Mora S, Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, pp 176–205

Roy CR, Gies HP, Tomlinson DW, Lugg DL (1994) Effects of ozone depletion on the ultraviolet radiation environment at the Australian stations in Antarctica. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurement and biological effects, vol 62. American Geophysical Union, Washington, DC, pp 1–15

Sancar A (1994) Mechanisms of DNA excision repair. Science 266:1954–1956

Sancar A (1996) No "end of history" for photolyases. Science 272:48–49

Sass L, Spetea C, Máté Z, Nagy F, Vass I (1997) Repair of UVB induced damage of photosystem II via *de novo* synthesis of the D1 and D2 reaction centre subunits in *Synechocystis* sp. PCC 6803. Photosynth Res 54:55–62

Scully NM, McQueen DJ, Lean DRS (1996) Hydrogen peroxide formation: the interaction of ultraviolet radiation and dissolved organic carbon in lake waters along a 43–75°N gradient. Limnol Oceanogr 41:540–548

Sheridan RP (2001) The role of ultraviolet radiation in maintaining the three-dimensional structure of a cyanobacterial mat community and facilitating nitrogen fixation. J Phycol 37:731–737

Shibata H, Katsuya B, Ochiai H (1991) Near-UV irradiation induces shock proteins in *Anacystis nidulans* R-2; possible role of active oxygen. Plant Cell Physiol 32:771–776

Shick JM, Dunlap WC (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol 64:223–262

Singh SP, Kumari S, Rostagi RP, Singh KL, Richa SRP (2010) Photoprotective and biotechnical potentials of cyanobacterial sheath pigment, scytonemin. Afr J Biotechnol 9:580–588

Sinha RP, Klisch M, Helbling EW, Häder D-P (2001) Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation. J Photochem Photobiol B 60:129–135

Smith RC, Baker KS (1981) Optical properties of the clearest natural seawaters. Appl Opt 20:177–188

Sobrino C, Ward ML, Neale PJ (2008) Acclimation to elevated carbon dioxide and ultraviolet radiation in the diatom *Thallasiosira pseudonana*: effects on growth, photosynthesis, and spectral sensitivity of photoinhibition. Limnol Oceanogr 53:494–505

Sorrels CM, Proteau PJ, Gerwick WH (2009) Organization, evolution, and expression analysis of the biosynthetic gene cluster for scytonemin, a cyanobacterial UV-absorbing pigment. Appl Environ Microbiol 75:4861–4869

Soule T, Stout V, Swingley WD, Meeks JC, Garcia-Pichel F (2007) Molecular genetics and genomic analysis of scytonemin biosynthesis in *Nostoc punctiforme* ATCC 29133. J Bacteriol 189:4465–4472

Soule T, Palmer K, Gao Q, Potrafka RM, Stout V, Garcia-Pichel F (2009a) A comparative genomics approach to understanding the biosynthesis of the sunscreen scytonemin in cyanobacteria. BMC Genomics 10:336–345

Soule T, Garcia-Pichel F, Stout V (2009b) Gene expression patterns associated with the biosynthesis of the sunscreen scytonemin in *Nostoc punctiforme* ATCC 29133 in response to UVA radiation. J Bacteriol 191:4639–4646

Tang EPY, Tremblay R, Vincent WF (1997) Cyanobacterial dominance of polar freshwater ecosystems: are high latitude mat formers adapted to low temperature? J Phycol 33:171–181

Tichy M, Vermaas W (1999) In vivo role of catalase-peroxidase in *Synechocystis* sp. strain PCC 6803. J Bacteriol 181:1875–1882

Van Baalen C (1968) The effects of ultraviolet irradiation on a coccoid blue-green alga: survival, photosynthesis, and photoreactivation. Plant Physiol 43:1689–1695

Van Baalen C, O'Donnell K (1972) Action spectra for ultraviolet killing and photoreactivation in the blue-green alga *Agmenellum quadruplicatum*. Photochem Photobiol 15:269–274

Vincent WF (1988) Microbial ecosystems of Antarctica. Cambridge University Press, Cambridge, 304 pp

Vincent WF (2000) Cyanobacterial dominance in the polar regions. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 321–340, 669 pp

Vincent WF, Neale PJ (2000) Mechanisms of UV damage to aquatic organisms. In: de Mora S, Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, pp 149–176

Vincent WF, Roy S (1993) Solar ultraviolet–B radiation and aquatic primary production: damage, protection, and recovery. Environ Rev 1:1–12

Vincent WF, Downes MT, Castenholz RW, Howard-Williams C (1993) Community structure and pigment organisation of cyanobacteria-dominated microbial mats in Antarctica. Eur J Phycol 28:213–221

Vincent WF, Mueller DR, Van Hove P, Howard-Williams C (2004) Glacial periods on early Earth and implications for the evolution of life. In: Seckbach J (ed) Origins: genesis, evolution and diversity of life. Kluwer Academic Publishers, Dordrecht, pp 481–501

Vinebrooke RD, Leavitt PR (1996) Effects of ultraviolet radiation on periphyton in an alpine lake. Limnol Oceanogr 41:1035–1040

Walker JJ, Pace NR (2007) Phylogenetic composition of Rocky Mountain endolithic microbial ecosystems. Appl Environ Microbiol 73:3497–3504

Walsby AE (1994) Gas vesicles. Microbiol Rev 58:94–144

Ward DM, Castenholz RW (2000) Cyanobacteria in geothermal habitats. In: Whitton BA, Potts M (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 7–59, 669 pp

Weisse T (1993) Dynamics of autotrophic picoplankton in marine and freshwater ecosystems. Adv Microb Ecol 13:327–370

Whitehead RF, de Mora SJ, Demers S (2000) Enhanced UV radiation – a new problem for the marine environment. In: de Mora S, Demers S, Vernet M (eds) The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, pp 1–34

Willis KJ, Bennett KD, Birks HJB (2009) Variability in thermal and UV-B energy fluxes through time and their influence on plant diversity and speciation. J Biogeogr 36:1630–1644

Wingard CE, Schiller JR, Castenholz RW (1997) Evidence regarding the possible role of c-phycoerythrin in ultraviolet-B tolerance in a thermophilic cyanobacterium. Photochem Photobiol 65:833–842

Wu H, Gao K, Villafañe VE, Watanabe T, Helbling EW (2005) Effects of solar UV radiation on morphology and photosynthesis of filamentous cyanobacterium *Arthrospira platensis*. Appl Environ Microbiol 71:5004–5013

Xing P, Hahn MW, Wu QL (2009) Low taxon richness of bacterioplankton in high-altitude lakes of the eastern Tibetan Plateau with a predominance of *Bacteroidetes* and *Synechococcus* spp. Appl Environ Microbiol 75:7017–7025

Zalar A, Tepfer D, Hoffmann SV, Kenney JM, Leach S (2007a) Directed exospermia: I. biological modes of resistance to UV light are implied through absorption spectroscopy of DNA and potential UV screens. Int J Astrobiol 6:229–240

Zalar A, Tepfer D, Hoffmann SV, Kollmann A, Leach S (2007b) Directed exospermia: II. VUV-UV spectroscopy of specialized UV screens, including plant flavonoids, suggests using metabolic engineering to improve survival in space. Int J Astrobiol 7:1–11

Part III

Molecular Ecology

Marine Picocyanobacteria

20

David J. Scanlan

Contents

Summary

Picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* numerically dominate vast tracts of the world's oceans and contribute a significant proportion of primary production, particularly in oligotrophic regions. The ecological success of these two genera suggests they possess sophisticated strategies to respond to variations in their environment. Indeed, it appears that it is the *in situ* community structure of these organisms which underlies this success, with the existence of specific ecotypes or lineages occupying different niches to populate the world's oceans. For *Prochlorococcus* there is now excellent physiological and genomic data for defining the basis of this niche partitioning particularly with respect to its vertical distribution down a water column. The situation for *Synechococcus* is more complex probably due to the larger spatial distribution of marine *Synechococcus* in oceanic ecosystems. This has led to extensive phylogenetic and physiological variation within the *Synechococcus* genus but the genomic basis for this phenotypic variation, and hence niche adaptation is less well understood. This chapter seeks to give an overview of the knowledge gained on these organisms over the last three decades focusing on aspects of ecology, physiology and molecular biology that are pertinent to this niche adaptation process.

20.1 Introduction

Whilst marine picocyanobacteria are now known to be the numerically dominant oxygenic phototrophs across vast tracts of the world ocean, it should not be forgotten that these organisms were only first documented around 30 years ago. Thus, it wasn't until epifluorescence microscopy of natural seawater samples showed the widespread occurrence of numerous orange autofluorescent cells that their presence was confirmed (Waterbury et al. 1979). We now know that this is a characteristic feature of most marine *Synechococcus* i.e. their possession of the accessory pigment phycoerythrin

D.J. Scanlan (✉)
School of Life Sciences, University of Warwick,
Gibbet Hill Road, Coventry CV4 7AL, UK
e-mail: D.J.Scanlan@warwick.ac.uk

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giving rise to a typical fluorescence signature in the 566–572 nm band range, that is distinct from the red fluorescence emission at 680 nm due to chlorophyll *a* (see e.g. Six et al. 2004). Around the same time Johnson and Sieburth (1979) performed ultrastructural studies on bacterial thin sections collected from the picoplankton size fraction in various oceanic locations. Their observations of 'type II' cells with peripheral thylakoids which were closely appressed to one another and a subsequent report of unknown chlorophyll *a* derivatives associated with particles <1 μm in size (Gieskes and Kraay 1983) were the first evidence for the existence of *Prochlorococcus*. The ensuing discovery of a flow cytometric 'signature' from abundant red-fluorescing cells deep in the euphotic zone (Chisholm et al. 1988) preceded their isolation into culture (Chisholm et al. 1992), and we now know members of the *Prochlorococcus* genus to be the most abundant phototrophs on Earth (Partensky et al. 1999b).

This chapter seeks to provide a broad overview of our current knowledge of these two genera, focusing on molecular ecological aspects, but readers are directed towards the many other excellent reviews that have been written on these organisms over the years (Glover 1985; Waterbury et al. 1986; Stockner 1988; Morel et al. 1993; Carr and Mann 1994; Partensky et al. 1999a, b; Hess et al. 2001; Scanlan and West 2002; Ting et al. 2002; Scanlan 2003; Garcia-Fernández et al. 2004; Hess 2004, 2008; Coleman and Chisholm 2007; Scanlan et al. 2009; Partensky and Garczarek 2010).

20.2 Marine *Synechococcus*

It should be remembered that the genus *Synechococcus* is polyphyletic (Honda et al. 1999; Robertson et al. 2001) including freshwater as well as marine strains. The marine *Synechococcus* lineage is phylogenetically 'clustered' on the basis of pigment composition (Six et al. 2007), salt requirement for growth, and G+C content (Waterbury and Rippka 1989). These clusters, originally defined as marine clusters A, B, and C (MC-A, MC-B, and MC-C) were reclassified (Herdman et al. 2001) so that MC-A and MC-B are now combined into two sub-clusters within *Synechococcus* cluster 5. MC-B (*Synechococcus* sub-cluster 5.2 but with strain PCC7001 removed, and mean DNA base composition ranging from 63% to 69% mol% G+C) contains mostly halotolerant strains isolated from coastal waters that possess phycocyanin but lack phycoerythrin (Fuller et al. 2003; Chen et al. 2006; Stomp et al. 2007; Haverkamp et al. 2009; Huang et al. 2012). It is *Synechococcus* sub-cluster 5.1 (≡ MC-A, mol% G+C = 55–62) however, which is the dominant *Synechococcus* group within the euphotic zone of open-ocean waters, comprising several taxonomically distinct clades (Fig. 20.1; Wood et al. 1998; Partensky et al. 1999a; Uysal 2000; Wilmotte et al. 2002; Fuller et al. 2003; Zwirglmaier et al. 2007, 2008; Huang et al. 2012). All members of this sub-cluster have elevated salt (Na^+, Cl^-, Mg^{++} and Ca^{++}) requirements for growth and contain phycoerythrin as their major light-harvesting pigment.

Phycoerythrin and phycocyanin together with associated chromophores form the rods of the phycobilisome, a structure connected to the photosystems via an allophycocyanin core. The phycobilisome is the major light-harvesting antennae of all marine *Synechococcus* strains (Fig. 20.2; Six et al. 2007). However, there is considerable spectral diversity in the phycoerythrins possessed by sub-cluster 5.1 strains. This is largely determined by the presence or absence of the chromophore phycourobilin (PUB), as well as the ratio of PUB to phycoerythrobilin (PEB) chromophores (Wood et al. 1985, 1999; Glazer 1999; Six et al. 2007; Blot et al. 2009; Scanlan et al. 2009). As a result, three major pigment types can be defined depending on the major phycobiliprotein found in the rods: phycocyanin (type 1), phycoerythrin I (type 2) or phycoerythrin II (type 3). Among strains containing both phycoerythrins I and II (i.e. type 3), four subtypes can be distinguished based on the ratio of the two chromophores (PUB:PEB) bound to these phycoerythrins, a ratio that can be low (type 3a), medium (type 3b), high (type 3c) or variable (type 3d) (Six et al. 2007). This latter type highlights that some members of *Synechococcus* subcluster 5.1 are capable of chromatic adaptation, so-called type IV chromatic adapters, being able to increase their PUB/PEB ratio under blue light compared with growth under green or white light (Palenik 2001; Everroad et al. 2006). Given that the wavelengths of maximal transmittance of light in blue, oligotrophic waters (~475 nm) and green, mesotrophic waters (525–550 nm) are close to the wavelengths of maximal absorption for PUB and PEB chromophores, it is perhaps not surprising that this variation in phycoerythrin chromophore content found in specific cultures is reflected in the distribution of natural marine *Synechococcus* populations. Thus, strains containing low PUB or PUB-lacking phycoerythrins have been shown to dominate in mesotrophic waters (classified optically as case 2 waters), whereas high PUB-containing phycoerythrin strains are more dominant in highly transparent, oligotrophic waters (case 1 waters) (Campbell and Iturriaga 1988; Olson et al. 1988, 1990a; Wood et al. 1998). In more turbid waters where red light prevails (particularly estuarine environments) 'green' (i.e. phycoerythrin-lacking, type 1) picocyanobacteria dominate (Chen et al. 2006; Stomp et al. 2007) whilst coastal seas of intermediate colouration can allow the co-existence of both phycoerythrin-containing and phycoerythrin-lacking strains (Stomp et al. 2004; Haverkamp et al. 2008, 2009). It is worth noting though, that given growth rates of chromatic adapters allow a change in cellular PUB:PEB ratio within 72 h, this could mean that some of these observed changes in the PUB/PEB ratio of phycoerythrins in bulk seawater may in part be due to physiological adaptation by these type 3d strains, in particular allowing flexibility in tracking changes in the relative availabilities of blue and green light

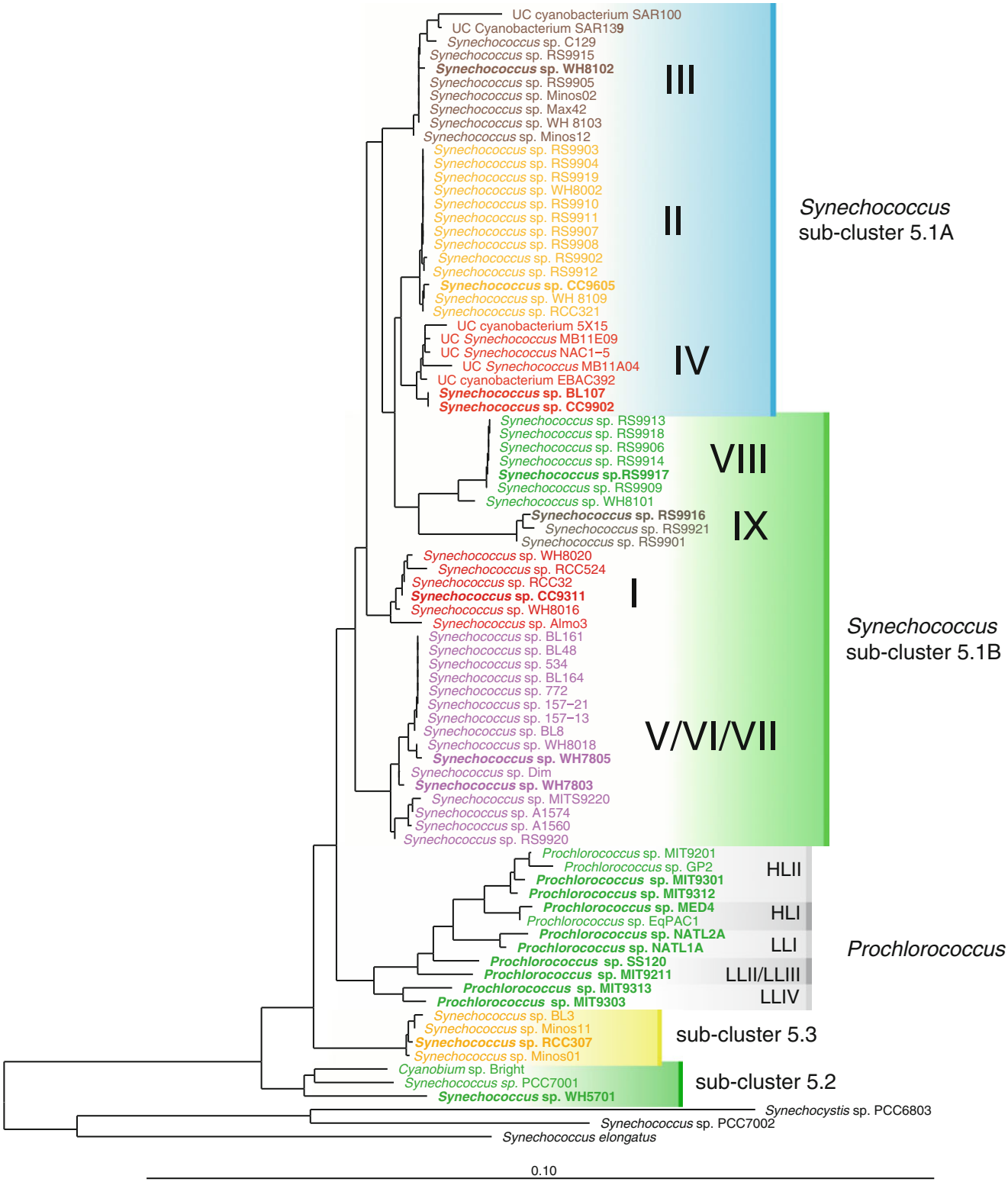

Fig. 20.1 Phylogenetic relationships amongst marine picocyanobacteria, using the 16S rRNA gene and a neighbour-joining algorithm. Strains with sequenced genomes are in *boldface*

that might occur following upwelling or mixing events in the open ocean (Everroad et al. 2006).

Although initial interest in picoplanktonic marine *Synechococcus* strains was largely due to their potential contribution to marine primary production, it is also unique features of their molecular biology and physiology, which separate them from well-characterised freshwater and euryhaline members of the genus, that is of significance to

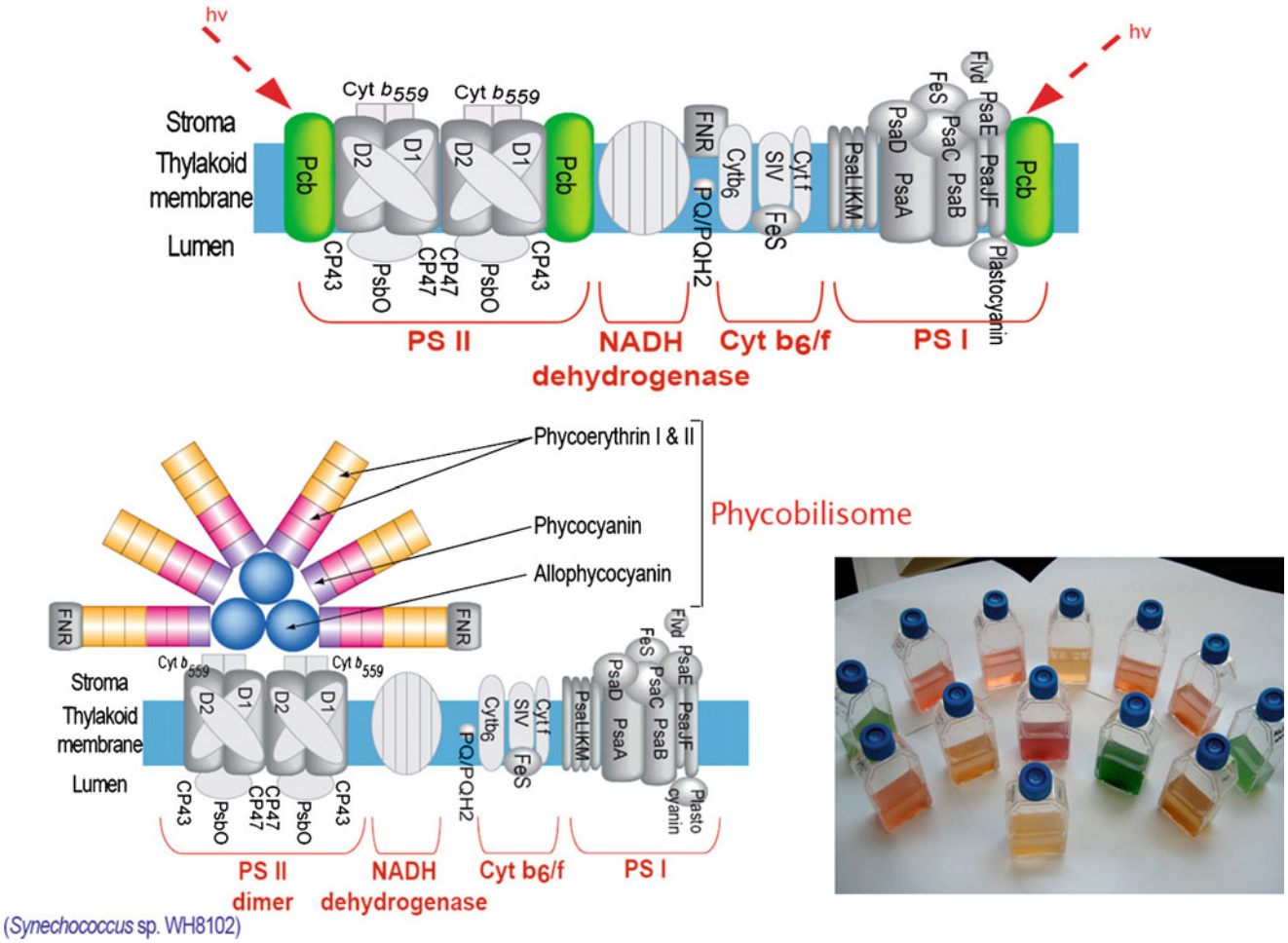

Fig. 20.2 Schematic representation of the photosynthetic light-harvesting machinery of *Prochlorococcus* (*upper panel*) and *Synechococcus* (*lower left panel*) (Images courtesy of Dr A. Dufresne). *Lower right panel*: diversity of pigment types amongst marine *Synechococcus* isolates (Image courtesy of Dr M. Ostrowski)

researchers. Thus, several strains are capable of a unique swimming motility (Brahamsha 1996a; McCarren et al. 2005; McCarren and Brahamsha 2005; Sect. 20.4), and most have the ability to acquire macro- and micro-nutrients at sub-micromolar concentrations found in oligotrophic open oceans. This has led to the development of specific regulatory circuits modifying nutrient transport processes, which are already known to be mechanistically different from those described in freshwater strains (Lindell et al. 1998; Wyman and Bird 2007) or are potentially unique (Ostrowski et al. 2010; Scanlan et al. 1997, 2009).

20.3 *Prochlorococcus*

Members of the *Prochlorococcus* lineage are characterised by the replacement of phycobilisomes with a chlorophyll-based antenna complex comprising thylakoid membrane proteins (Pcb's) binding unique divinyl derivatives of chlorophyll *a* and *b* (Fig. 20.2) (Goericke and Repeta 1992; LaRoche et al. 1996; Garczarek et al. 2000, 2001; Bibby et al. 2003). Photosystem I-specific and photosystem II-specific variants of these chlorophyll-binding Pcb proteins have evolved (Partensky and Garczarek 2003), a feature also found in *Prochlorothrix hollandica* and *Prochloron didemni*. These latter species, together with *Prochlorococcus* were initially classified among the "Prochlorophyta". Use of this term is now invalid with the clear demonstration that these three groups position on separate branches within the cyanobacterial radiation and that the ability to synthesize chlorophyll *b* and bind it in antenna complexes has evolved independently several times (Urbach et al. 1992; Palenik and Haselkorn 1992; Ting et al. 2002). Further phylogenetic studies have shown that the *Prochlorococcus* genus is closely related to marine *Synechococcus* sub-cluster 5.1, forming sister clades, but with a rapid diversification of these two groups from a common phycobilisome-containing ancestor (Urbach et al. 1998; Fig. 20.1). However, both natural populations and cultured *Prochlorococcus* isolates still produce phycoerythrin, though only a small amount per cell (Hess

et al. 1996; Steglich et al. 2001, 2003a) and with a most likely function as a photoreceptor (Steglich et al. 2005).

Natural *Prochlorococcus* communities comprise multiple populations, so-called high light (HL) and low light (LL)-adapted ecotypes, a feature initially suggested by the frequent observations of (i) bimodal red fluorescence distributions (dim and bright populations) revealed by flow cytometry (ii) by the increase in chlorophyll fluorescence per cell (Campbell and Vaulot 1993) and (iii) the high divinyl-chlorophyll (DV chl) *b*/DV chl *a* ratio of deep *Prochlorococcus* populations (Veldhuis and Kraay 1993; Partensky et al. 1996). Culture studies have subsequently shown that LL-adapted strains possess a higher DV-chl *b*/DV-chl *a* ratio, their optimal level of growth irradiance is lower, and they become photoinhibited at irradiances at which the HL-adapted strains grow maximally (Partensky et al. 1993; Moore et al. 1998; Moore and Chisholm 1999; Sects. 20.7 and 20.10). Molecular systematic studies show the HL and LL-adapted ecotypes to be phylogenetically distinct (Fig. 20.1; Moore et al. 1998; Urbach et al. 1998; West and Scanlan 1999; Rocap et al. 2002) but with members of the HL cluster being the most recently evolved. A key feature of the *Prochlorococcus* genus is that most lineages (i.e. with the exception of the LLIV clade) contain strains that have undergone significant genome reduction and specialisation (Dufresne et al. 2005; Kettler et al. 2007; Partensky and Garczarek 2010; Sect. 20.9) a facet presumably tightly linked to their occupation of specific niches. LL ecotypes are genetically more diverse than their HL counterparts (Zinser et al. 2006; Martiny et al. 2009a), which likely reflects the availability of several different niches at the bottom of the euphotic zone and distinct evolutionary adaptation to the available light and nutrient fields. Whilst the use of HL- and LL-adapted ecotype terminology has largely proved coherent with molecular ecological studies of community structure (see Sect. 20.7), it should be noted that the LLI clade comprises strains that appear to occupy intermediate positions in the water column (Zinser et al. 2007), or at least a more variable niche than HL/'true' LL counterparts, and hence likely represents a new 'phototype' (Partensky and Garczarek 2010; see also Sect. 20.7).

As well as fundamental differences in light harvesting apparatus between members of the *Prochlorococcus* and *Synechococcus* genera, giving rise to pigment differences that underlie the light qualities they optimally collect (Ting et al. 2002), there are also concomitant differences in cell size. *Synechococcus* cells range between 0.6 and 1.6 μm in diameter (Waterbury et al. 1986). For *Prochlorococcus*, HL strains are roughly 0.7 μm diameter spheres whereas LL strains, particularly members of the LLIV lineage, can be larger and more oval (Morel et al. 1993; Lichtlé et al. 1995; Rippka et al. 2000; Ting et al. 2007; Sect. 20.4). This is consistent with an increase in cell size seen in natural *Prochlorococcus* populations down a water column (Sieracki et al. 1995). These differences in cell size have knock-on effects both with respect to differences in the elemental composition of the two genera (Bertilsson et al. 2003; Heldal et al. 2003) and in nutrient assimilation capacity, with the smaller *Prochlorococcus* cells having a higher surface area to volume ratio and hence being potentially diffusion limited at lower nutrient concentrations than *Synechococcus* (Chisholm 1992).

20.4 Morphology

A relatively large number of marine picocyanobacterial clones have now been isolated into culture (Vaulot et al. 2004), allowing the extent of morphological variation between and within the two genera to be described (Perkins et al. 1981; Kursar et al. 1981; Waterbury et al. 1986; Chisholm et al. 1992; Ting et al. 2007). The possession of closely appressed photosynthetic thylakoid membranes is the primary ultrastructural feature that distinguishes *Prochlorococcus* from *Synechococcus* (Chisholm et al. 1992). Within *Prochlorococcus*, thylakoid membranes appear to form complete concentric rings around the cell periphery or are horse-shoe shaped depending on the strain analysed (Lichtlé et al. 1995). Moreover, a less extensive intracytoplasmic (photosynthetic) membrane system is seen in *Prochlorococcus* sp. MED4, a HLI strain, compared to *Prochlorococcus* sp. MIT9313, a LLIV strain, elegantly shown by cryo-electron tomography (Fig. 20.3, Ting et al. 2007) and entirely consistent with the discrete niches occupied by the two ecotypes. These latter two *Prochlorococcus* strains interestingly appear to contain an extensive network of interconnected intracytoplasmic membranes. The channels linking the luminal space of adjacent intracytoplasmic membranes are not abundant, but it has been proposed they could contribute to maintaining a uniform electrochemical potential across the intracytoplasmic lamellae (Ting et al. 2007).

Regarding cell surface properties, a feature relevant to how picocyanobacterial cells are perceived by the 'outside world' and particularly by protist grazers and/or bacteriophage, cyanobacterial cell walls are structurally similar to those of gram-negative bacteria but sharing some of the characteristics of gram-positive bacteria (Hoiczyk and Hansel 2000). In *Prochlorococcus* sp. MIT9313, the cell wall is approximately 34 nm thick, comprising an outer membrane and distinct peptidoglycan layer, the latter approximately 4 nm thick (Ting et al. 2007). This is considerably less than the approximately 15–16 nm thickness of the peptidoglycan layer reported in marine *Synechococcus* spp. WH8102/WH8113 (Samuel et al. 2001; Ting et al. 2007), though another report suggests that the peptidoglycan layer of WH8102 is thinner at around 6 nm (McCarren et al. 2005). In the HLI *Prochlorococcus* sp. MED4, cell wall thickness is reduced by about a half compared to the MIT9313 cell wall,

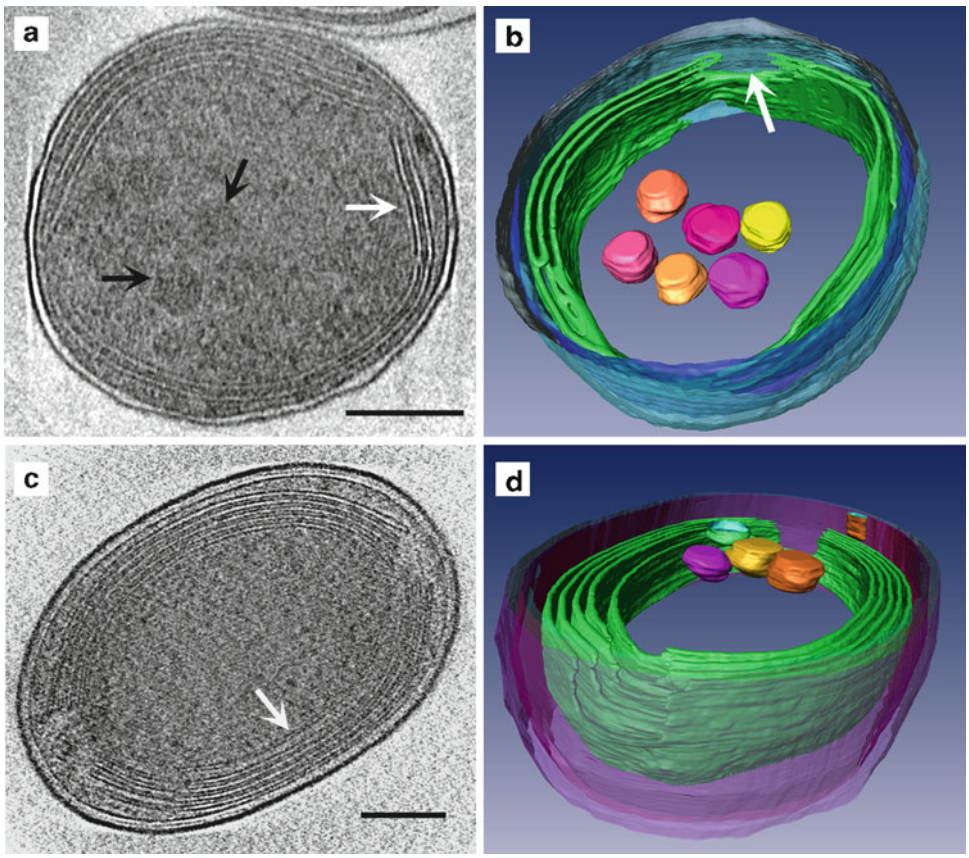

Fig. 20.3 Cellular architecture of *Prochlorococcus*. (**a**) 1.8-nm-thick tomographic slice of a *Prochlorococcus* sp. MED4 cell. Two to three bands of intracytoplasmic (photosynthetic) lamellae are visible near the cell periphery (*white arrow*). Several polygonal structures resembling carboxysomes are clustered in the central cytoplasmic space (*black arrows*). (**b**) Surface-rendered model of *Prochlorococcus* sp. MED4 showing that there are specific regions (*white arrow*) where the intracytoplasmic membranes (*green*) terminate, resulting in the formation of large gaps in the membrane bands. Note that an additional carboxysome is visible when the *Prochlorococcus* sp. MED4 cell is viewed from this perspective. Modeled 3D structures include the outer membrane (*light blue*), inner membrane (*dark blue*), intracytoplasmic lamellae (*green*), and carboxysomes (*yellow, pink, orange*). (**c**) A 1.8-nm-thick tomographic slice of a *Prochlorococcus* sp. MIT9313 cell. (**d**) Side view of a surface-rendered model of *Prochlorococcus* sp. MIT9313, in which the regions where the intracytoplasmic membranes (*green*) terminate at the cell poles are visible. Note that these fenestrations in the intracytoplasmic membranes create areas of direct contact between the central cytoplasmic space and the region between the cell membrane and outermost intracytoplasmic membrane. Bars = 200 nm. Structures depicted include the cell wall (*purple, pink, blue*), extensive intracytoplasmic membrane system (*green*), and carboxysomes (*yellow, pink, orange, blue*). Tomographic images and surface-rendered models courtesy of Dr Claire S. Ting (Adapted from Ting et al. (2007). Reprinted with permission of the publisher)

due partly to a thinner outer membrane but also by a reduced periplasmic space and the apparent absence of a prominent peptidoglycan layer, which when visible is less dense and narrower. Such differences may be due to the cost of synthesising a prominent peptidoglycan layer, particularly in open ocean surface waters where nitrogen, an important structural component of the peptide cross-links and N-acetyl groups, may be limiting (Ting et al. 2007). At first glance this reduction in cell wall 'strength' appears paradoxical for a bacterium that needs to resist relatively high ocean salinities. However, marine picocyanobacteria adopt the well-known strategy of accumulating high (molar) amounts of compatible solutes to acclimate to high salt concentrations. In particular, the compatible solute glucosylglycerate (GGA) is found in high amounts in *Prochlorococcus* (Klähn et al. 2010). As an additional benefit, since GGA has a negative charge it has been suggested that it can replace glutamate to counterbalance the charge of those ions e.g. K^+ and Na^+ that are abundant in salt-loaded microbial cells. Given the high amounts of glutamate required to compensate for the positive charge of such inorganic ions, utilisation of the N-free compound GGA is potentially a clever mechanism by which *Prochlorococcus* further reduces its N demand (Klähn et al. 2010).

The cell envelope of marine *Synechococcus* also has multiple layers (Fig. 20.4) but which, in addition to inner and outer membranes and peptidoglycan layers, at least in motile strains, contains an additional surface layer. This surface

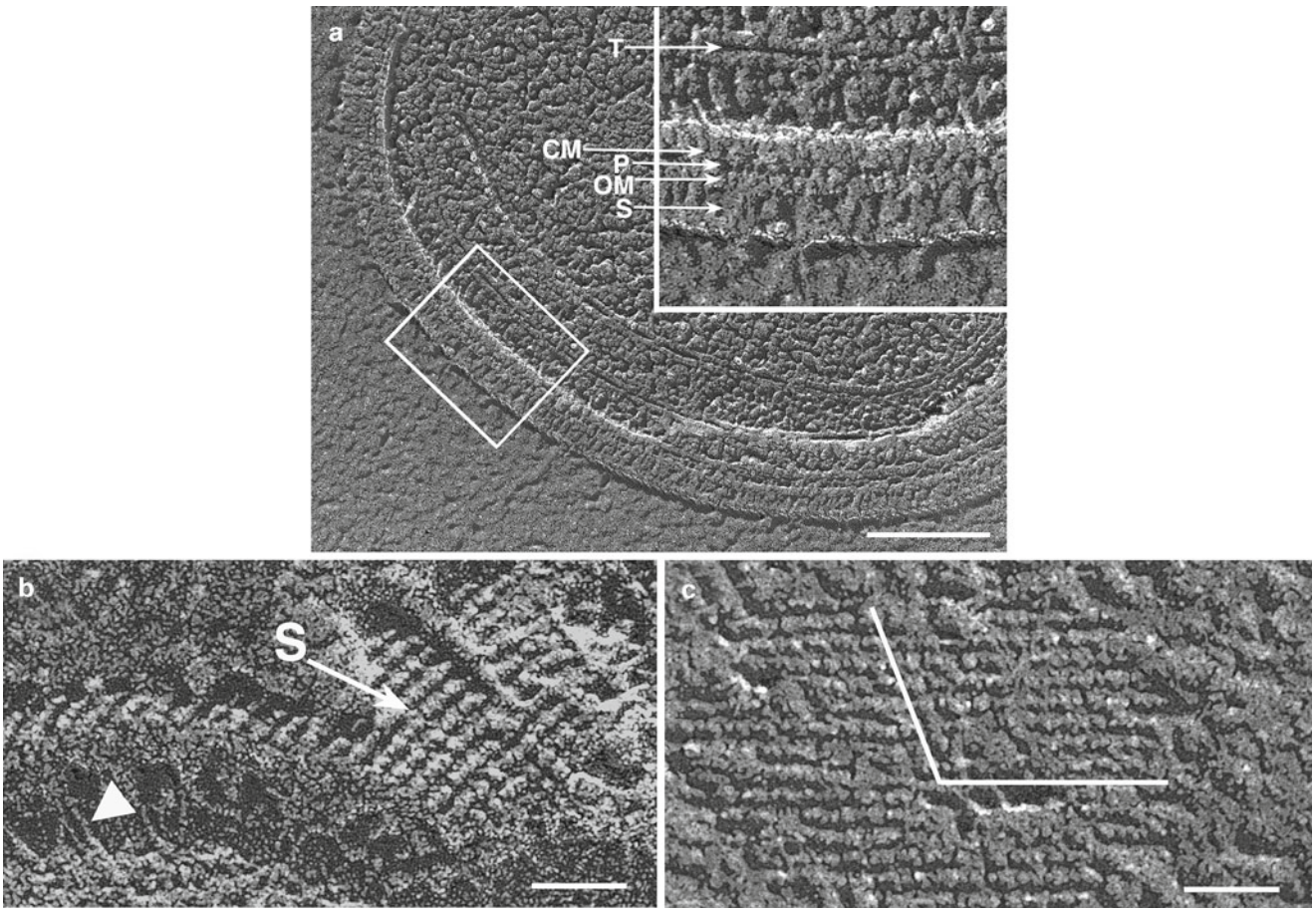

Fig. 20.4 Cell envelope structure of *Synechococcus* sp. WH8113. (**a**) Cross fracture revealing concentric layers of cell envelope. The inset corresponds to the outlined section of cell envelope comprising cell membrane (*CM*), peptidoglycan layer (*P*), outer membrane (*OM*), and surface layer (*S*). A thylakoid layer (*T*) is also indicated. Scale bar, 200 nm. (**b**) Crystalline outer surface of the surface layer (*S*) revealed where ambient ice is broken away. The *arrowhead* indicates a fibre of 5 nm thickness that arises from the cell membrane and extends to higher layers. Scale bar, 50 nm. (**c**) Patch of crystalline outer surface near the fracture plane revealed by etching ambient ice. Scale bar, 50nm (*From* Samuel et al. (2001). Reprinted with permission of the authors)

layer is approximately 35 nm thick in *Synechococcus* sp. WH8113, and just over 20 nm wide in WH8102 (McCarren et al. 2005). This outer layer demonstrates properties of an S-layer (Ŝmarda et al. 2002) forming a paracrystalline lattice over the cell surface (Fig. 20.4). In *Synechococcus* sp. WH8113 short fibrils (5 nm wide and 150 nm long), or spicules, have been observed emanating from the cell surface (Samuel et al. 2001). It was subsequently proposed that that these spicules, which are believed to span the entire cell envelope, may be able to transmit motion in the cytoplasmic or outer membrane to the surrounding medium (Samuel et al. 2001) and hence form a mechanistic basis for the swimming motility observed in some marine *Synechococcus* strains (Waterbury et al. 1985; Brahamsha 1999), a property in fact restricted to members of clade III within sub-cluster 5.1 (Toledo et al. 1999). However, in another motile strain, *Synechococcus* sp. WH8102, spicules have never been observed on the cell surface suggesting such structures, if present, are not required for motility in all swimming *Synechococcus* strains. Instead, WH8102 cells contain an 8 nm thick outermost S-layer separated from the outer membrane by a 14-nm space in which there are apparent columnar connections between the continuous S-layer and the outer membrane surface. Indeed, fractures removing the S-layer reveal a layer of fibrillar material. Interestingly, a mutant in a single gene, *swmA*, lacks the paracrystalline S-layer (McCarren et al. 2005) suggesting that it is the SwmA protein that forms the entire S-layer. The *swmA* mutant is non-motile suggesting that the S-layer is directly, or indirectly (as a site of attachment for other components) required for swimming ability. Indeed, another cell-surface located protein, SwmB, has also been shown to be required for motility (McCarren and Brahamsha 2007). This protein has an irregular, punctuate distribution rather than the homogeneous S-layer formed by SwmA. SwmB is a 'giant protein', predicted to be >1 MDa in size and has a highly repetitive structure.

It has been proposed that this latter feature may be important for interaction with the highly repetitive S layer formed by SwmA which, if such an interaction occurs, could cause conformational changes in SwmB resulting in structural changes in the S layer that produce localized contractions that generate thrust for swimming (McCarren and Brahamsha 2007).

Other cell surface appendages have been reported for two *Synechococcus* isolates isolated from off the mouth of the Chesapeake Bay. These appendages resemble spinae, forming hollow tubes 44–65 nm in diameter and as long as 2.7 μm (Perkins et al. 1981). Interestingly, in a freshwater *Cyanobium* sp. similar structures can be induced in cultures subject to protozoan grazing, production of spinae being accompanied by cell aggregation and microclony formation, suggesting a causal connection between these processes (Jezberová and Komárková 2007).

Biochemical characterisation of the lipopolysaccharide (LPS) component of the outer membrane layer has recently been reported in marine *Synechococcus*. Strains typical of either coastal (*Synechococcus* sp. CC9311) or open-ocean environments (*Synechococcus* sp. WH8102) were examined and shown to produce similar, very simplified LPS structures lacking typical components of bacterial LPS, such as Kdo (3-deoxy-D-*manno*-octulosonic acid), heptose, and phosphate (Snyder et al. 2009). Interestingly, specific differences in LPS composition were observed between the two *Synechococcus* strains, features that may give rise to differential phage infection or recognition by grazers or that may impart differences in nutritional quality to *Synechococcus* strains that may be important for heterotrophic grazers that consume them.

20.5 Abundance and Distribution of Marine Picocyanobacteria

Studies of picophytoplankton community structure as well as many ancillary measurements often collected on research cruises are now available for a wide variety of oceanic regimes comprising both horizontal transects encompassing large spatial scales e.g. the Atlantic Meridional Transect (AMT) (Zubkov et al. 2000; Heywood et al. 2006), as well as single site temporal studies. Examples of the latter include the long-running Bermuda Atlantic time series (BATS) in the western North Atlantic (Durand et al. 2001; Steinberg et al. 2001) as well as the Hawaii ocean time series (HOT) at station ALOHA in the Pacific (Karl and Lukas 1996; Campbell and Vaulot 1993; Campbell et al. 1994, 1997; Karl et al. 2001). Whilst *Prochlorococcus* abundance generally greatly exceeds that of *Synechococcus* in areas where they co-occur, a major exception includes regions seasonally or permanently enriched with nutrients via upwellings or coastal inputs which allows *Synechococcus* to proliferate (Partensky et al. 1999a). Probably the highest *Synechococcus* abundances have been observed in the Costa Rica upwelling dome, a region of high primary production due to physical forcing resulting in upwelling of nutrient-rich waters. Numbers at this location range between 1.5 and 3.7×10^6 cells mL^{-1} (Li et al. 1983; Saito et al. 2005) markedly higher than other oceanic environments (see below) and are unusual since *Synechococcus,* and unicellular marine cyanobacteria in general, are not typically associated with blooming conditions. It has been proposed that it is the unique chemical signature of the Costa Rica dome (including complexation of cobalt, cadmium and copper and relatively low iron and zinc concentrations) that precludes diatom (or other fast-growing eukaryotic phytoplankton) bloom formation in this region (Saito et al. 2005). Elsewhere, high *Synechococcus* cell abundances have been reported in coastal waters off west Africa ($1–5.2 \times 10^5$ cells ml^{-1}; Partensky et al. 1996; Zubkov et al. 2000; Zwirglmaier et al. 2007), the Arabian Sea ($4.5–6.7 \times 10^5$ cells ml^{-1}; Burkill et al. 1993; Fuller et al. 2006), in French Polynesian coral atoll lagoon waters (3.7×10^5 cells ml^{-1}, Charpy and Blanchot 1998) and in Woods Hole Harbour, Massachusetts (3.6×10^5 cells ml^{-1}, Waterbury et al. 1979). Such numbers are one or more orders of magnitude higher than those reported for other open ocean environments (e.g. maximal values at BATS of $3.3–5.6 \times 10^4$ cells ml^{-1}; Goericke and Welschmeyer 1993; Campbell et al. 1994; Durand et al. 2001) and particularly the nutrient depleted oligotrophic gyres ($1–4 \times 10^3$ cells ml^{-1}; Partensky et al. 1999a; Zubkov et al. 2000; Heywood et al. 2006; Zwirglmaier et al. 2007). Indeed, a belt of high summer *Synechococcus* abundance appears to mark the boundary between temperate/mesotrophic and oligotrophic waters around 44–47°N along the 20°W meridian in the North Atlantic, whilst peak concentrations of *Synechococcus* occur more consistently in the southern Atlantic at about 37–39°S in both austral spring and autumn (Zubkov et al. 2000). This belt of high abundance of *Synechococcus* occurred in waters at about 16°C in both hemispheres, irrespective of the season.

As a consequence of the wide distribution of *Synechococcus* in marine waters, it is present in waters covering a wide temperature range, from ca. 2–3°C to >30°C (Waterbury et al. 1986; Shapiro and Haugen 1988; Fuller et al. 2006). However, in the lower range (<14°C), temperature is clearly an important discriminator of *Synechococcus* abundance (Li 1998), as shown by the several orders of magnitude decline in cell number between 44°S and 62°S in the South Atlantic Ocean, numbers ranging from <10 cells ml^{-1} in −1°C waters to $1.6–3.4 \times 10^4$ cells ml^{-1} in waters between 9°C and 14°C (Marchant et al. 1987; see also Odate and Fukuchi 1995; Fouilland et al. 1999; Wilmotte et al. 2002). A similar abundance decrease has been reported along transects in the North Atlantic Ocean, between the central gyre and waters near Greenland or Iceland (Murphy and Haugen 1985; Cottrell et al. 2008).

A conspicuous departure from this trend, are the relatively high cell numbers (10^5–10^6 cells ml^{-1}) that can be found in the Baltic Sea (Kuosa 1991; Andersson et al. 1994). In truly polar seas picocyanobacteria are typically observed in negligible or low abundance (Gradinger and Lenz 1989, 1995), whilst higher numbers (e.g. see Garneau et al. 2006) have been attributed to input from freshwater sources (Waleron et al. 2007). The low abundance of picocyanobacteria in polar seas has been ascribed to the cold-tolerant but not psychrophilic growth characteristics of high-latitude cyanobacteria (Tang and Vincent 1999) and their inability to keep pace with loss processes such as advection and grazing in cold oceans (Vincent et al. 2000). However, a recent study in coastal waters of the Chukchi Sea and the Beaufort Sea in the Arctic Ocean which showed *Synechococcus* abundance (which averaged $4–8 \times 10^4$ cells L^{-1}) did not differ significantly between winter and summer (where temperatures averaged 4.1°C in summer and −1.8°C in winter) suggests that *Synechococcus* can sustain sufficient growth throughout the winter to balance mortality (Cottrell and Kirchman 2009). Here, DOM consumption in the dark has been implicated to be important to the survival of *Synechococcus* during winter darkness (Cottrell and Kirchman 2009).

Whilst most surveys of *Synechococcus* abundance across horizontal space have been conducted over relatively long distance intervals, a study in the Celtic Sea (Martin et al. 2005) showed striking variability in the *Synechococcus* population which fluctuated more than 60-fold (between 2.5×10^3 and 1.5×10^5 cells ml^{-1}) over the continuous 7-day sampling period. This included incredibly abrupt changes in cell concentration: early on 11 July 2004 the population changed from 5×10^3 cells ml^{-1} to in excess of 9×10^4 cells ml^{-1} in just 96 min, equivalent to a 50-fold change in abundance in just under 12 km. Often, dramatic increases in abundance were reversed over a similar length-scale, giving rise to extreme spikes in abundance. The high resolution of the data (each spike typically comprising ten or more data points) suggests such spikes are genuine features of *Synechococcus* distribution patterns. Subsequent modelling studies implicate grazing by heterotrophic protists as a key parameter required to reproduce the witnessed degree of variability in *Synechococcus* abundance, though dependent on sub-kilometre seawater mixing values (Martin et al. 2008).

In contrast to *Synechococcus*, *Prochlorococcus* distribution patterns are more restricted, the genus being largely confined to a latitudinal belt of the world's oceans roughly bounded by the latitudes 45°N to 40°S (Partensky et al. 1999a, b; Buck et al. 1996; Johnson et al. 2006; Zwirglmaier et al. 2007). Within this region, that mainly encompasses open ocean oligotrophic waters but also extends into warm, mesotrophic stratified areas, *Prochlorococcus* is exceedingly abundant regularly reaching concentrations over 1×10^5 cells ml^{-1} (Olson et al. 1990b; Campbell and Vaulot 1993; Zubkov et al. 2000; Johnson et al. 2006; Zwirglmaier et al. 2007, 2008). *Prochlorococcus* thus dominates *Synechococcus* numerically in these open ocean environments, leading to discrete peaks in abundance of the two genera across horizontal transects that encompass both temperate/mesotrophic/upwelling waters favoured by *Synechococcus,* compared to oligotrophic/stratified waters favoured by *Prochlorococcus* (Fig. 20.5). That said, *Prochlorococcus* can also be found in some coastal areas, such as the plume of the Rhone river in the Mediterranean Sea (Vaulot et al. 1990; Joux et al. 2005) and in Japanese waters of Suruga Bay (Shimada et al. 1995). However, it is not clear whether *Prochlorococcus* grows actively in such environments or is simply advected from oceanic waters (Partensky et al. 1999a).

Prochlorococcus cell abundance above and below the 45°N to 40°S latitudes declines fairly rapidly. Thus, along a transect in the eastern North Atlantic between 5°S and 61°N *Prochlorococcus* mean abundance dropped from 1.83×10^5 cells ml^{-1} between 5°S and 24°N to 7.3×10^4 cells ml^{-1} between 25°N and 45°N and then to 6.8×10^3 cells ml^{-1} between 50°N and 61°N (Buck et al. 1996). Interestingly, the dramatic shift between *Prochlorococcus*- and *Synechococcus*-dominated surface waters across the South Atlantic Subtropical Front (35–45°S) is not mirrored by a decline in *Prochlorococcus* metabolism, using methionine uptake as a proxy of cellular metabolic activity (Zubkov and Tarran 2005). Rather, temperature appears to be a critical factor in dictating the distribution of members of the *Prochlorococus* genus (Partensky et al. 1999a; Bouman et al. 2006; Johnson et al. 2006; Zwirglmaier et al. 2008) consistent with culture studies (Zinser et al. 2007) but with distinct differences between ecotypes (Sect. 20.7). Thus, waters of ca 10°C appear to mark the lower limit where *Prochlorococcus* has been recorded (Buck et al. 1996; Zubkov et al. 2000), whilst at the opposite end of the scale, waters >30°C can also retain relatively large numbers e.g. 1×10^5 cells ml^{-1} in surface waters of the Arabian Sea reaching 31.5°C (Fuller et al. 2006). However, maximum integrated abundances occur between 26°C and 28°C and decrease above this temperature (Partensky et al. 1999a). Water column stability also appears to be a critical factor with deep winter mixing leading to marked *Prochlorococcus* abundance minima and sometimes complete loss from the water column (Olson et al. 1990b; Lindell and Post 1995; DuRand et al. 2001; Malmstrom et al. 2010). Hence, at BATS in March–April following winter mixing, *Synechococcus* concentrations approach or exceed those of *Prochlorococcus* (DuRand et al. 2001). This is coincident with maximal surface nitrate concentrations in February–March and has been explained by the long held belief that *Prochlorococcus* cannot use nitrate for growth (Moore et al. 2002). This inability to assimilate nitrate has also been used to partly explain the low abundance of *Prochlorococcus* relative to *Synechococcus* in coastal,

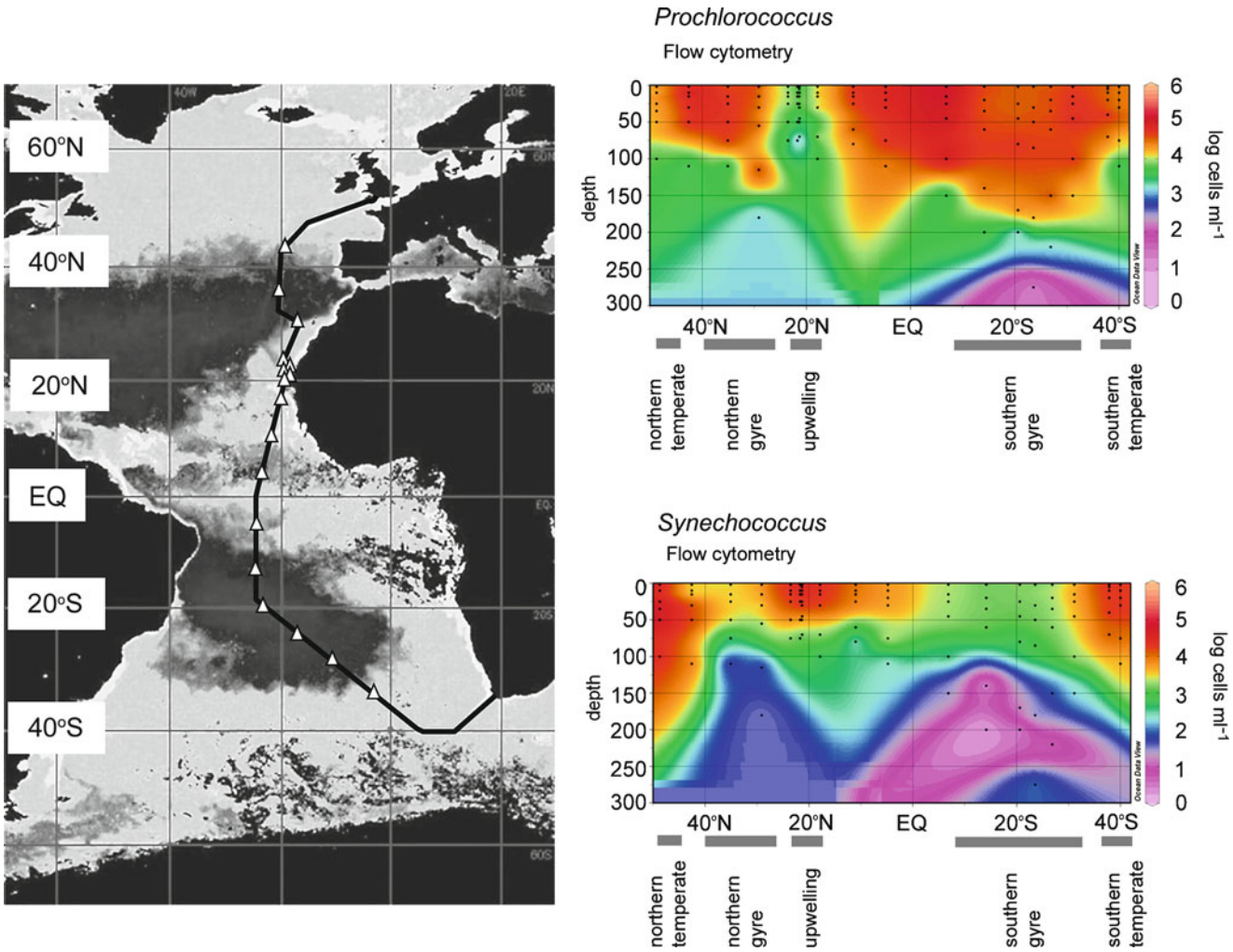

Fig. 20.5 Basin-scale distribution patterns of marine picocyanobacteria along an Atlantic Meridional Transect. The cruise track (*left panel*), and *Prochlorococcus* (*upper right panel*) and *Synechococcus* (*lower right panel*) abundance derived from flow cytomtery data, are indicated (Adapted from Zwirglmaier et al. (2007). Reprinted with permission of the publisher)

temperate, and upwelling regions (Blanchot and Rodier 1996; Partensky et al. 1996; Saito et al. 2005), including the aforementioned Costa Rica dome region where high nitrate concentrations are present even in surface waters. However, recent analysis of metagenomic sequence data has added a new twist to this story with genes for both nitrate and nitrite assimilation being distributed amongst microdiverse *Prochlorococcus* lineages, and expressed *in situ* (Martiny et al. 2009b; Sect. 20.10). Martiny et al., reconcile these observed field distributions by suggesting that (i) *Synechococcus* has a higher growth rate at elevated nitrate concentrations, and hence dominates in nitrate-rich waters whilst (ii) *Prochlorococcus* has a higher uptake rate at very low nitrate concentrations due to their small size (i.e., lower Ks) and hence dominates in nitrate poor regions e.g. the surface mixed-layer at lower latitudes. *Synechococcus* also predominates over *Prochlorococcus* in the mixed layer under stratified conditions in the subtropical north Atlantic. This has been attributed to copper toxicity associated with photodegradation of organic ligands that otherwise would lower copper bioavailability, and a higher copper sensitivity in *Prochlorococcus* than *Synechococcus* (Mann et al. 2002).

Vertical distribution patterns of marine picocyanobacteria down a water column have been categorised into three main types (Partensky et al. 1999b) (i) where *Prochlorococcus* is confined to the surface mixed layer, and numbers drop quickly below the thermocline (Fig. 20.6a), but occasionally with a small peak in abundance localized at the thermocline (Fig. 20.6b). In such, usually nearshore, waters the *Synechococcus* distribution pattern is similar, with comparable or slightly higher numbers; (ii) a sharp peak in *Prochlorococcus* abundance occurs near the bottom of the

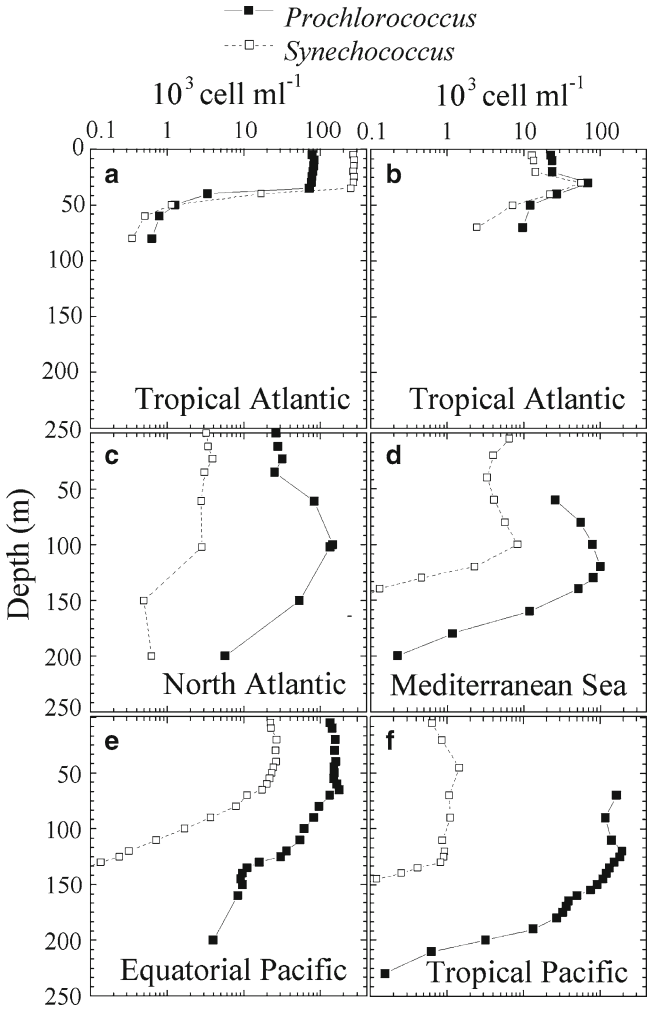

Fig. 20.6 Typical vertical distribution patterns of *Prochlorococcus* and *Synechococcus*. (**a** and **b**) Surface layer maximum. (**c** and **d**) Deep maximum. (**e** and **f**) Uniform distribution over the entire euphotic zone. (**a** and **b**) North tropical Atlantic, EUMELI3 cruise. (**a**) EU site, off the coast of Mauritania (20°N, 18°W). (**b**) MESO site (18°N, 21°W). (**c**) North Atlantic 30°N, 23°W (11). (**d**) Eastern Mediterranean Sea, 34°N 18°E, MINOS cruise. (**e**) Equatorial Pacific 150°W, 5°S (174). (**f**) Tropical Pacific 150°W, 16°S. (**d** and **f**) *Prochlorococcus* chlorophyll fluorescence was too weak to be detected at the surface (*From* Partensky et al. (1999b). Reprinted with permission of the authors and publisher)

euphotic zone, decreasing by an order of magnitude at the surface (Fig. 20.6c, d) (iii) *Prochlorococcus* extends from surface waters to the bottom of the euphotic zone in similar numbers, though with numbers reducing at extreme depths, and with *Synechococcus* abundance one to two orders of magnitude lower (Fig. 20.6e, f). This third profile type is widespread in oceanic waters, encompassing waters roughly between 30°S and 30°N where the water column is never mixed beyond 100–150 m deep and varies only slightly throughout the year. *Prochlorococcus* populations thus generally extend much deeper than *Synechococcus*, with members of the latter genus often disappearing below 150 m.

20.6 Contribution to Primary Production and Carbon Cycling

The numerical abundance of marine picocyanobacteria suggests that these organisms are major contributors to oceanic primary production. However, whilst there is a plethora of literature on size-fractionated primary production measurements (e.g. see Marañón et al. 2000, 2001 and references therein) there is little information on the contribution of specific taxonomic groups within such fractions, which encompass not only *Prochlorococcus* and *Synechococcus* within the <3 μm portion, but also photosynthetic eukaryotes. The exception is the seminal study by Li (1994) which used $NaH^{14}CO_3$ incorporation into phytoplankton and subsequent flow cytometric sorting to attribute CO_2 fixation to specific groups at three locations in the North Atlantic. Using such an approach *Prochlorococcus* was shown to contribute 57% of total production at one site at the base of the euphotic zone whilst at a more productive surface location the contribution of *Prochlorococcus* (11%) and *Synechococcus* (20%) was comparable. Recently, Jardillier et al. (2010) undertook a similar study, this time encompassing 18 stations in the sub-tropical North Atlantic located between 12–26°N and 24–36°W. Averaged across all stations *Prochlorococcus*, due largely to its high abundance, contributed 45 ± 17% to CO_2 fixation despite low cell-specific rates (1.2 ± 0.6 fg C cell^{-1} h^{-1}) whilst *Synechococcus*, with moderate abundances and cell-specific CO_2 fixation rates (9.5 ± 4.3 fg C cell^{-1} h^{-1}) contributed 21 ± 13%. Combined CO_2 fixation rates then, are consistent with these marine picocyanobacterial groups being major contributors to oceanic CO_2 fixation but with further basin-scale studies clearly needed to more accurately map the contribution of these taxa at larger scales and across a range of habitats. The importance of this is magnified by the predicted increase in ocean stratification and the decrease in nutrient supply due to climate change, a scenario thought to favour small phytoplankton at the expense of diatoms (Bopp et al. 2005). Indeed, Polovina et al. (2008) have recently demonstrated an expansion of oceanic areas exhibiting the lowest chlorophyll concentrations (<0.07 μg.L^{-1}) at annual rates varying between 0.8% and 4.3%, depending on gyres. Whilst this may reduce the global productivity of the oceans it has been proposed that this may consequently induce an increase in the relative contribution of *Prochlorococcus* to this global productivity, largely as a consequence of the increased extent of their northern/southern distribution (Partensky and Garczarek 2010). This has been predicted to result in less-efficient shallow export production and hence reduce ocean carbon sequestration, which would greatly amplify the positive feedback of climate change (Buesseler et al. 2007). This prediction is based on the assumption that all picoplankton production is cycled through the microbial

loop and that none sinks from the euphotic zone directly. However, an alternative view suggests that despite their small size, picoplankton may contribute more to oceanic carbon export than is currently recognized, either via direct cell aggregation and export of picoplankton as POC, or mesozooplankton/large filter feeder–mediated sinking of picoplankton-based production (Richardson and Jackson 2007). Further work is necessary to reconcile this.

20.7 Picocyanobacterial Community Structure *In Situ*

Studies of the genetic structure of natural picocyanobacterial populations suggest that, at least for *Prochlorococcus,* its success in colonising the entire euphotic zone is largely due to the differential distribution of different *Prochlorococcus* ecotypes. Thus, *in situ* studies using the 16S rRNA (Moore et al. 1998; West and Scanlan 1999; West et al. 2001), 16S–23S rRNA internal transcribed spacer (ITS) (Johnson et al. 2006; Zinser et al. 2006; Malmstrom et al. 2010), the DNA-dependent RNA polymerase *rpoC1* (Ferris and Palenik 1998; Jameson et al. 2010), *cpeB* (Steglich et al. 2003b), and *rbcL* (Wawrik et al. 2003) gene markers show that the HL- and LL-adapted ecotypes exhibit particular depth-dependent distributions (Fig. 20.7). The HL group, which can be further subdivided into HLI and HLII ecotypes (these have also been termed eMED4 and eMIT9312, respectively, where the prefix "e" indicates "ecotype" and the strain name following that is the type strain from which it was named; Ahlgren et al. 2006), is generally restricted to surface waters in stratified water columns (see Fig. 20.7). However, in the Red Sea, deep winter mixing reduces numbers of LL ecotypes such that HLII genotypes can subsequently dominate the *Prochlorococcus* population even at depth (see West et al. 2001; Fuller et al. 2005). Within the LL-adapted ecotypes there is also evidence of specific partitioning 'at depth' of particular ecotypes (e.g. see Steglich et al. 2003b; Fuller et al. 2006; Zinser et al. 2006). Thus, even within strains that appear mostly confined to low light conditions, there appears to be the potential for partitioning within the water column. This existence of defined ecotypes with distinct physiologies is likely to permit survival of the population as a whole over a much broader range of light and nutrient conditions (i.e. defined niches) than could be possible by a single homogeneous population (Moore et al. 1998). Interestingly though, as eluded to above, recent molecular ecological work has added further complexity to the HL/LL paradigm. Thus, members of the LLI ecotype (eNATL2A) have been found at moderate levels of abundance in warm stratified waters just below the thermocline, and in surface waters, whilst at higher latitudes in more mixed waters they co-occur with HLI ecotypes down the entire euphotic zone (Johnson et al. 2006).

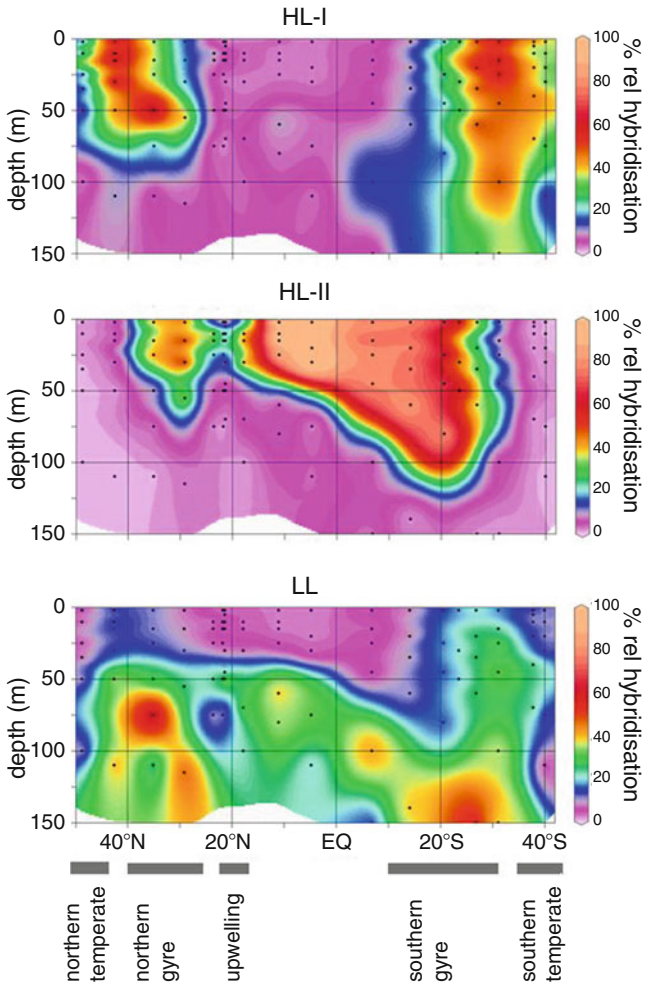

Fig. 20.7 Basin-scale distribution patterns of specific *Prochlorococcus* ecotypes along an Atlantic Meridional Transect. The presented *dot blot* hybridization data describes the distribution of HLI (*upper panel*), HLII (*middle panel*) and LL (*lower panel*) *Prochlorococcus* ecotypes along the AMT15 cruise track. *Coloured* contours indicate % relative hybridization (specific probe hybridisation as a proportion of all PCR products amplified by primers OXY107F and OXY1313R). The *y*-axis indicates depth in metres. *Black dots* represent sampling points (*From* Zwirglmaier et al. (2007). Reprinted with permission of the publisher)

It is possible that members of the LLI ecotype 'merely' have a greater ability to tolerate the temporary exposure to high light at or near the surface during turbulent mixing (Zinser et al. 2007). Such an idea is consistent with a recent culture study that shows the LLI type strain (NATL2A) can withstand temporary light shock better than the LLII strain SS120 (Malmstrom et al. 2010). Alternatively, given that genomic properties of NATL2A share features of both HL and LL strains (Sect. 20.10) and that LLI strains are phylogenetically the closest branch to the HL lineage, eNATL2A may represent a new 'phototype' (Partensky and Garczarek 2010) with properties intermediate between 'classic' HL and LL ecotypes.

Analyses of the distribution patterns of specific *Prochlorococcus* ecotypes across several ocean basins

(Johnson et al. 2006; Bouman et al. 2006, 2011; Zinser et al. 2007; Zwirglmaier et al. 2007, 2008; Huang et al. 2012) has provided more detailed insight into the factors that are important in dictating *Prochlorococcus* population structure. Certainly, at the level of taxonomic resolution of the 16S rRNA or ITS region, temperature, irradiance and mixing intensity are important determinants of the observed distribution patterns of the different taxa. This is consistent with HLI genotypes dominating more weakly stratified surface waters at temperate latitudes (35–48°N and 35–40°S), whilst HLII genotypes are most prevalent in strongly stratified subtropical and tropical regions (30°N–30°S), although there is some overlap at the transition between temperate and subtropical zones (Zwirglmaier et al. 2008). For members of the HLII ecotype a direct effect of temperature on the growth rate of cultured strains has been demonstrated, with the lower temperature limit for growth of cultured HLII strains, 16°C, corresponding to a drastic decline in field abundance. Above this threshold, growth rate and field abundance both increase with increasing temperature, and the temperature at which growth rates of cultures are maximal (ca. 24–26°C) corresponds to maximal field abundances (Zinser et al. 2007). HLI strains show the greatest cold tolerance, followed by LLI, HLII and LLIV ecotypes, in that order. These differences in the growth of strains below 15°C correlate well with the observed habitat ranges of individual ecotypes in higher latitude, colder waters of an Atlantic Meridional Transect (Johnson et al. 2006) suggesting that ecotype-specific geographic ranges are determined by genetically defined cold temperature tolerances (Zinser et al. 2007). Whilst maximum reported abundances of the HLII ecotype correspond well with their optimal temperature for growth, maximum reported abundances of HLI, LLI and (particularly) LLIV ecotypes are at temperatures below their growth optima. It has been proposed that at this optimal temperature range, enhanced competition (e.g. from HLII ecotypes) or predation could be the overriding factor in determining abundance of HLI and LLI ecotypes, dominating over physiology (Zinser et al. 2007).

Clone library construction using the ITS marker has revealed a plethora of new LL sequences, distinct from the four known LL ecotypes, for which molecular ecological data is currently lacking (Zinser et al. 2006; Martiny et al. 2009a; Lavin et al. 2010). These include a novel cluster, termed NC1, comprising sequences from the Atlantic and Pacific Ocean (Martiny et al. 2009b) as well as two potentially novel lineages from an oxygen minimum zone region in the eastern tropical Pacific (Lavin et al. 2010). Similar molecular approaches have also recently uncovered several novel HL clusters (Huang et al. 2012) including a lineage comprising two-sub-clusters that appears prevalent in high nutrient low chlorophyll (HNLC) waters (Rusch et al. 2010; West et al. 2001). This HNLC clade has a relatively restricted distribution compared to the HLI and HLII ecotypes, seemingly prevalent in regions of high ammonium but low iron availability (see section 20.10).

Using available sequence information, Martiny et al. (2009a) have shown that, depending on the sequence similarity cut-offs that are used to define a taxon or operational taxonomic unit (ranging from ≥80% to ≥99.5% similarity), the number of shared sequences increases between oceanic regions at broad definitions, whereas more unique types are found in a given region at finer cut-offs. In other words there appears to be high local microdiversity at the fine taxonomic scale, whereas broader phylogenetic groups are more cosmopolitan. This local microdiversity may be due to the large population size and relatively short generation time of *Prochlorococcus*, cells evolving faster than ocean currents can mix them (Martiny et al. 2009a). As an extension to this work, these authors point to a strong hierarchical association between environmental parameters and phylogenetic depth. Thus, light intensity is an important determinant of community structure when taxa are defined at broad resolution, temperature is important at intermediate level resolution whilst P-availability does not explain community structure, regardless of taxonomic resolution (see also Martiny et al. 2006). Furthermore, nitrate availability explains some of the variability in *Prochlorococcus* community structure when taxa are defined with fine resolution, consistent with field and genetic evidence that natural *Prochlorococcus* populations have the capability to utilize nitrate (Casey et al. 2007; Martiny et al. 2009b).

Regarding *Synechococcus*, various molecular markers show that genetic diversity within the sub-cluster 5.1 lineage is also extensive (reviewed in Scanlan and West 2002), i.e. based on the 16S rRNA gene (Fuller et al. 2003; Choi and Noh 2009; Fig. 20.1), the ITS region (Rocap et al. 2002; Ahlgren and Rocap 2006; Choi and Noh 2009; Jing et al. 2009a, b; Mella-Flores et al. 2011; Huang et al. 2012), *rpoC1* (Toledo and Palenik 1997; Toledo et al. 1999; Mühling et al. 2005, 2006) *narB* (Jenkins et al. 2006; Paerl et al. 2008), *ntcA* (Penno et al. 2006) and *petB* (Mazard et al. 2012). Such studies suggest that around twenty discrete clades can be assigned. However, it is unclear whether such clades describe species or ecotypes, or indeed some intermediate phylogenetic level (Dufresne et al. 2008; Sect. 20.9). Certainly, the only physiological trait that appears to be clade specific, such that it might delineate an ecotype, is motility, which resides only within members of clade III (Toledo et al. 1999). However, it appears that not all clade III members are motile, though it is possible that these are motile strains that have lost the ability to swim (Fuller et al. 2003). In contrast, pigmentation patterns generally cannot be used as a key characteristic to differentiate clades, consistent with the presence of different pigment types within the same clade (Palenik 2001; Ahlgren and Rocap 2006; Haverkamp et al. 2008; Choi and Noh 2009), and with the lack of concordance between phycobilisome rod and core genome phylogenies (Six et al.

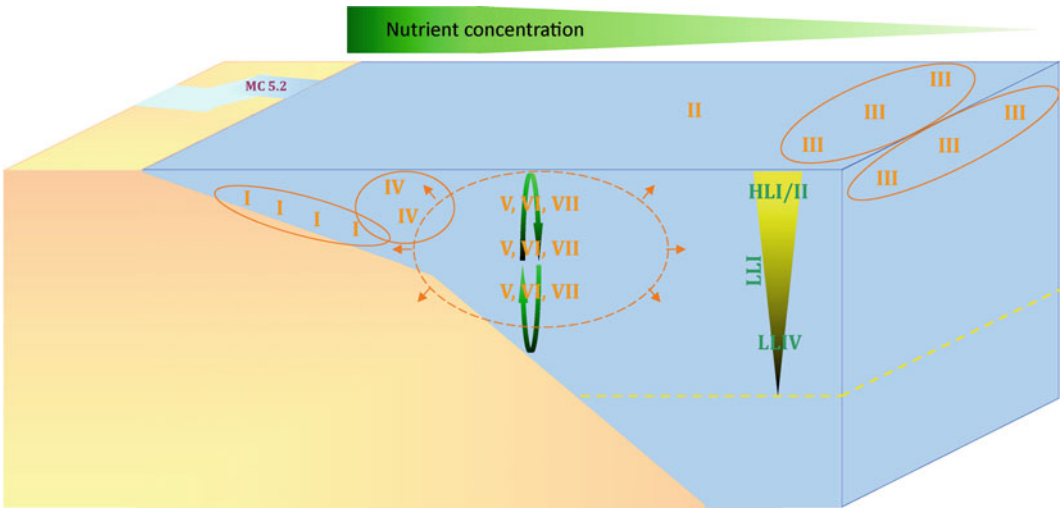

Fig. 20.8 Ecological distribution patterns of marine picocyanobacterial lineages. The *yellow arrow* indicates the light gradient and the *bottom* of the uphotic zone is indicated by a *dashed yellow line*. The *green bar* indicates regions of differing nutrient concentration spanning eutropic to oligotrophic waters. A region of upwelling is depicted by a *green arrow*. *Prochlorococcus* ecotypes are indicated in *green*; *Synechococcus* clades are indicated in *orange* (Image courtesy of Dr S. Mazard)

2007). Thus far however, all cultured clade VIII members appear to lack phycoerythrin i.e. are of pigment type 1 (see Fuller et al. 2003).

Using a suite of 16S rDNA oligonucleotide probes specific to the ten individual clades described by Fuller et al. (2003) and dot blot hybridisation technology, recent progress has been made in analysing the dynamics of *Synechococcus* community structure at the genetic level and at different spatial and temporal scales (Fuller et al. 2006; Zwirglmaier et al. 2007, 2008). Thus, along transects in the Mediterranean Sea, Indian, eastern South Pacific and Atlantic Oceans, distinct spatial changes in *Synechococcus* population structure have been observed, these changes correlating well with changes in water column conditions i.e. between coastal, open-ocean mesotrophic and open-ocean oligotrophic waters (Zwirglmaier et al. 2007, 2008). Hence, at least for the four most abundant lineages (clades I-IV), there is becoming a clearer picture of their ecological preferences, i.e. the ecological conditions under which they most frequently appear (Fig. 20.8). Clades I and IV, which often co-occur, are generally confined to high latitude coastal and/or temperate mesotrophic waters roughly above 30°N/30°S (Brown and Fuhrman 2005; Brown et al. 2005; Zwirglmaier et al. 2007, 2008; Jing et al. 2009a; Tai and Palenik 2009; Paerl et al. 2011). In contrast, members of clade II appear more abundant in the subtropical and tropical waters, usually in coastal/continental shelf zones (Zwirglmaier et al. 2007, 2008) but also in offshore, oligotrophic environments (Toledo and Palenik 2003; Ahlgren and Rocap 2006). Such distributions may at least in part be driven by temperature since scatter plots of clade I and IV relative hybridization values against temperature (Fig. 20.9) shows the temperature range for clades I and IV to be very similar and with waters between 7°C and 17°C offering the highest relative hybridisation values. This contrasts with the clearly higher temperature profile of *Synechococcus* clade II (Fig. 20.9). Members of clade III show no obvious latitudinal (or temperature) preference but rather are most prevalent in ultraoligotrophic open-ocean waters. Finally, whilst clade V/VI/VII genotypes appear to have a relatively wide distribution but in low abundance, there are exceptions, with such genotypes dominating mesotrophic upwelling waters off the coast of Oman in the Arabian Sea (Fuller et al. 2006). A caveat here though, is that the distribution patterns of these latter lineages is largely based on dot blot hybridisation data using an oligonucleotide probe that targets these clades collectively. Similarly, ITS clone libraries constructed from the Costa Rica dome upwelling region specifically show a dominance of clade VII members in this region (Saito et al. 2005). The relatively broad brush picture of clade distributions we have so far will be aided in the future by further use of quantitative PCR (qPCR) studies to allow more sensitive detection of the low abundance clades, specific *rpoC1* probes for clades I-IV having been recently developed (Tai and Palenik 2009). Indeed, use of the latter qPCR strategy applied to a 3-year time series of surface samples at a coastal site in the northeast Pacific Ocean demonstrated evidence of a seasonal cycle for the two dominant clades, I and IV. At this location, clade IV genotypes were typically more abundant but those from clade I dominated during periods prior to the annual peak in total *Synechococcus* (Tai and Palenik 2009). A key next step will be deciphering the relative importance of genetic versus environmental factors driving these clade-specific changes in *Synechococcus* abundance.

That the distribution patterns for specific *Synechococcus* lineages are evident over large spatial scales potentially sug-

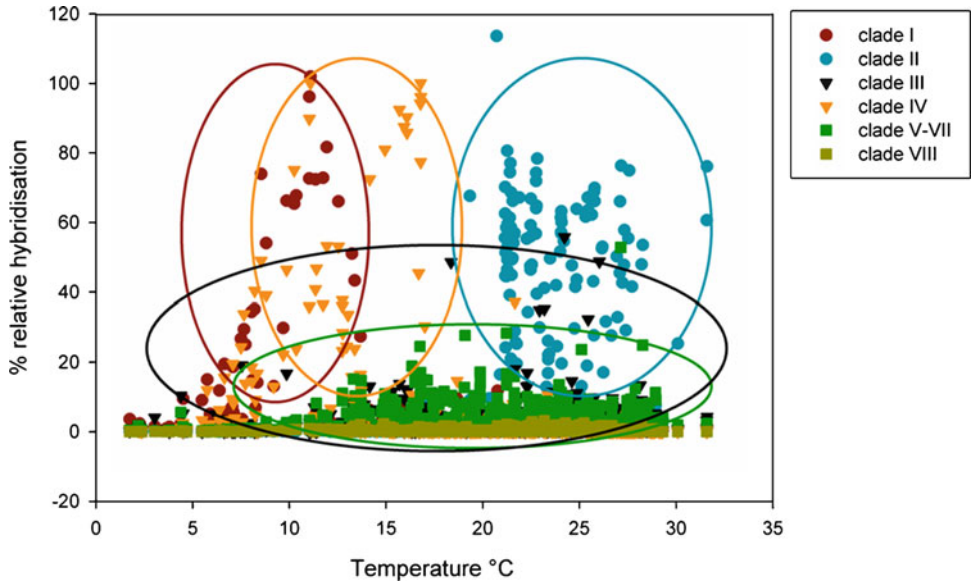

Fig. 20.9 Scatter plot showing the relative abundance of specific *Synechococcus* clades ($n=526$; data from percentage relative hybridization values) as a function of seawater temperature (*From* Zwirglmaier et al. (2008). Reprinted with permission of the publisher)

gests that understanding the factors important in the niche partitioning process at the ocean basin scale (e.g. an Atlantic Meridional Transect) can ultimately be extended to the global ocean. However, recent analysis (Zwirglmaier et al. 2008) performed using multivariate statistics show strikingly for both genera, that a large part (48% and 64% for *Prochlorococcus* and *Synechococcus* respectively) of the observed patterns in community structure remain unexplained by the abiotic parameters that are most commonly considered when attempting to explain spatial partitioning patterns (e.g. the macronutrients N and P and physical factors such as irradiance or temperature). Whilst some of this might be explained by the hierarchical relationship between environmental parameters and phylogenetic depth alluded to above, it also suggests that other, biotic, factors such as grazing (Zwirglmaier et al. 2009) and phage infection (Mühling et al. 2005) play an important role in defining individual niche space (see below and this Chap. 21). Indeed, biotic and abiotic factors may not be mutually exclusive given that copper stress has been shown to cause induction of a temperate marine cyanophage (Sode et al. 1997) while phosphate limitation has been implicated in inducing pseudolysogeny in marine *Synechococcus*, in which a phage-infected cell grows and divides even though its virus is pursuing a lytic infection (Mann 2003).

20.8 Factors Controlling Marine Picocyanobacterial Abundance *In Situ*

Assessing the factors that cause variation in picocyanobacterial growth rates and/or growth yield *in situ* is not straightforward. As described above, differences in the distribution patterns of *Synechococcus* and *Prochlorococcus* emphasise that although the same controlling factors are being encountered, either abiotic (irradiance, temperature, water column stability, nutrients etc.) or biotic (grazing, viral infection, toxins), these are being responded to differently by each genus. Not only that, but there are also obvious differential responses to the same parameter even between members of the same genus. Indeed, the fact that *Synechococcus* isolates from the same clade can have different specific growth rates adds further complexity to this problem (Palenik 2000). In the natural environment however, it is the 'population' specific growth rate that is determined and most work on controlling factors is hence targeted at this level.

In situ growth rates of marine picocyanobacteria have been obtained using a variety of methods including techniques that are unaffected by grazing mortality (e.g. analysis of the fractions of cells in specific cell cycle stages or pigment labelling), or those where independent measurements of grazing mortality are made (e.g. by dilution culture or addition of selective inhibitors) to adjust measured net growth rates (see Furnas and Crosbie 1999 for review). Using such approaches, the bulk of picocyanobacterial growth rates approach one doubling per day (0.7 day^{-1}). However, *Synechococcus* growth rates >1.5 day^{-1} (over 2 doublings per day) have been measured in near-surface populations in coastal or shelf waters (Furnas 1991). For *Prochlorococcus*, maximal growth rates have been reported from the mixed layer of equatorial upwelling zones and the monsoonal Arabian Sea (Vaulot et al. 1995; Shalapyonok et al. 1998; Liu et al. 1998). Noteworthy, is that picoplankton growth rates do not seem to differ significantly even in oligotrophic regions (Liu et al. 1995).

Experimental perturbation of natural systems via nutrient enrichment experiments does hint though, towards growth

constraint by nutrient limitation in some regions. Thus, during the iron enrichment experiment, IronEx II, in the eastern equatorial Pacific *Prochlorococcus* cell size and chlorophyll *a* per cell both increased, implying cells were iron limited (Mann and Chisholm 2000). Indeed, cell division rates increased from 0.6 to 1.1 day^{-1} during the 6 days of exposure to the elevated iron concentrations in the patch. In contrast, *Prochlorococcus* mortality rates virtually doubled after the addition of iron, maintaining a relatively constant population size (Mann and Chisholm 2000). Thus, whilst cell division rates appear significantly iron limited in this region, biomass is constrained both by iron limitation and microzooplankton grazing. However, in similar high nutrient, low chlorophyll waters *Synechococcus* does not appear to be strongly iron-limited (Wells et al. 1994). In the oligotrophic subtropical North Atlantic macronutrients appear to play a greater role with N the proximal limiting nutrient for chlorophyll synthesis and carbon fixation but also evidence of specific co-limitation, by N and P, of *Synechococcus* nucleic acid synthesis and growth (Moore et al. 2008). Similarly, in the tropical North Atlantic, *Prochlorococcus* and *Synechococcus* pigment biosynthesis appears N-limited but with picocyanobacterial cell abundance only increasing following combined N/P or N/Fe addition (Davey et al. 2008). Variability in the response to macronutrient addition is also evident, with *Synechococcus* abundance co-limited by N and P in the Gulf of Aqaba, whilst *Prochlorococcus* did not respond to nutrient additions (Mackey et al. 2009).

Mortality factors, largely grazing and viral infection (Chap. 21), also play an important role in controlling picophytoplankton abundance *in situ*. Although more work is required to fully address the relative importance of each factor, or indeed, interactions between these processes, recent experimental data suggests that grazing dominates viral lysis of these organisms (Baudoux et al. 2007, 2008; Kimmance et al. 2007). However, no consistent picture has yet emerged regarding protistan grazing on marine picocyanobacteria with some studies showing high mortality rates e.g. values for *Prochlorococcus* and *Synechococcus* ranging from 20–116% to 43–87% of growth rates (Liu et al. 1995) or up to 50% removal of the cyanobacterial assemblage per day (Caron et al. 1991; Worden and Binder 2003) whilst other studies suggest *Synechococcus* and *Prochlorococcus* are less susceptible to grazing than heterotrophic bacteria. Thus, although a wide variety of protists have been shown to graze marine picocyanobacteria, including both heterotrophic (e.g. see Christaki et al. 1999, 2002; Guillou et al. 2001) and mixotrophic (Frias-Lopez et al. 2009) organisms, two amoebae species favoured six different bacterial prey species over *Synechococcus*, the latter being the only prey that did not support growth of the predator (Pickup et al. 2007). Similar grazing experiments with three species of heterotrophic nanoflagellates (HNFs) showed *Synechococcus* cells were ingested but not digested by the grazer and thus did not support growth, whereas the heterotrophic bacteria in their study did (Boenigk et al. 2001). Further, Christaki and colleagues found *Synechococcus* and *Prochlorococcus* to be grazed by both HNFs (Christaki et al. 2002) and ciliates (Christaki et al. 1999) but only at a low level, and with ciliates showing a clear preference for *Synechococcus* over *Prochlorococcus*. Recent attempts to resolve some of these discordances suggests that at least for *Synechococcus*, prey susceptibility to grazing is manifest at the individual strain level (Zwirglmaier et al. 2009; Apple et al. 2011). Indeed, prey selectivity seems to be a two-step process, occurring both at the stage of ingestion, and digestion. At present, the molecular basis of such selectivity is unclear but cell surface properties appear to be important, as evidenced by the fact that a phage-resistant mutant of *Synechococcus* sp WH7803 possessing a modified LPS layer is grazed preferentially over the wild type strain (Zwirglmaier et al. 2009), whilst a *swmB* mutant of *Synechococcus* sp. WH8102, encoding a 'giant' cell surface localised protein, is also grazed at higher rates than wild type (Strom et al. 2012). Given that a key feature of marine picocyanobacterial genomes is the presence of 'island' regions which show deviations in tetranucleotide frequency compared with the core genome (Coleman et al. 2006; Sect. 20.9; Dufresne et al. 2008; Sect. 20.9) and that within these island regions enzymes involved in carbohydrate modification are abundant e.g. glycosyltransferases, glycoside hydrolases and other enzymes involved in carbohydrate modification, the above grazing data strengthens the suggestion that these cell envelope modifying genes change cell surface characteristics to aid grazer evasion (Palenik et al. 2003; Jones et al. 2006). Moreover, the fact that such genes are located in potentially 'dynamic' and variable genomic island regions hints at a fascinating biological arms race between predator and prey.

20.9 Genomics

Over 20 marine picocyanobacterial genomes are now publically available and with several others in progress (Table 20.1). Genome size is characteristically small in these organisms e.g. *Prochlorococcus* genomes range in size from 1.64 to 2.7 Mb (Kettler et al. 2007) and *Synechococcus* between 2.2 and 2.86 Mb (Dufresne et al. 2008), compared to the average size of other sequenced cyanobacteria (5.33 ± 3.69 Mb) (Scanlan et al. 2009). Indeed, most *Prochlorococcus* genomes are smaller than 2 Mb, the exception being members of the LLIV lineage, e.g. *Prochlorococcus* sp. MIT9313 and MIT9303, these latter strains also demonstrating a slightly larger size than other *Prochlorococcus* (Ting et al. 2007) suggesting that cell volume is tightly linked to genome size (Partensky and Garczarek 2010). For the remaining

Table 20.1 Summary of published *Prochlorococcus* and *Synechococcus* genomes[a]

Genus/strain	Sub-cluster or ecotype	Clade number	Genome size (Mb)	No. of protein -coding genes	GC%	Status and accession numbers	References
Synechococcus							
CC9311	5.1B[b]	I[c]	2.61	2,892	52	[C]:CP000435[d]	Palenik et al. (2006)
CC9605	5.1A[b]	II[c]	2.51	2,645	59	[C]:CP000110[d]	Dufresne et al. (2008)
WH8102	5.1A[b]	III[c]	2.43	2,519	59	[C]:BX548020[d]	Palenik et al. (2003)
CC9902	5.1A[b]	IV[c]	2.23	2,358	54	[C]:CP000097[d]	Dufresne et al. (2008)
BL107	5.1A[b]	IV[c]	2.28	2,553	54	[WGS]:AATZ00000000[e]	Dufresne et al. (2008)
WH7803	5.1B[b]	V[c]	2,37	2,586	60	[C]:CT971583[d]	Dufresne et al. (2008)
WH7805	5.1B[b]	VI[c]	2.62	2,934	57	[WGS]:AAOK00000000[e]	Dufresne et al. (2008)
RS9917	5.1B[b]	VIII[c]	2.58	2,820	65	[WGS]:AANP00000000[e]	Dufresne et al. (2008)
RS9916	5.1B[b]	IX[c]	2.66	3,009	60	[WGS]:AAUA00000000[e]	Dufresne et al. (2008)
WH5701	5.2	–	2.86	3,129	66	[WGS]:AANO00000000[e]	Dufresne et al. (2008)
RCC307	5.3	–	2.22	2,583	61	[C]:CT978603[d]	Dufresne et al. (2008)
Prochlorococcus							
MED4	HL	HLI[f]	1.66	1,929	31	[C]:BX548174[d]	Kettler et al. (2007)
MIT9515	HL	HLI[f]	1.70	1,908	31	[C]:CP000552[d]	Kettler et al. (2007)
MIT9301	HL	HLII[f]	1.64	1,907	31	[C]:CP000576[d]	Kettler et al. (2007)
AS9601	HL	HLII[f]	1.67	1,926	31	[C]:CP000551[d]	Kettler et al. (2007)
MIT9215	HL	HLII[f]	1.74	1,989	31	[C]:CP000825[d]	Kettler et al. (2007)
MIT9312	HL	HLII[f]	1.71	1,962	31	[C]:CP000111[d]	Kettler et al. (2007)
NATL1A	LL	LLI[f]	1.86	2,201	35	[C]:CP000553[d]	Kettler et al. (2007)
NATL2A	LL	LLI[f]	1.84	2,158	35	[C]:CP000095[d]	Kettler et al. (2007)
SS120	LL	LLII[f]	1.75	1,925	36	[C]:AE017126[d]	Kettler et al. (2007)
MIT9211	LL	LLIII[f]	1.69	1,855	38	[C]:CP000878[d]	Kettler et al. (2007)
MIT9303	LL	LLIV[f]	2.68	3,022	50	[C]:CP000554[d]	Kettler et al. (2007)
MIT9313	LL	LLIV[f]	2.41	2,843	51	[C]:BX548175[d]	Kettler et al. (2007)

[a]Several other marine *Synechococcus* and *Prochlorococcus* genomes have been recently completed or are in progress (see http://camera.calit2.net/microgenome/)
[b]Sub-cluster number as defined in Dufresne et al. (2008)
[c]Clade number as defined in Fuller et al. (2003)
[d][C]: complete genome sequence
[e][WGS] incomplete genome sequences (estimated coverage >99.8%)
[f]Ecotype number as defined in Kettler et al. (2007)

Prochlorococcus lineages, extensive genome streamlining has occurred concomitant with a pronounced reduction in G+C content (Dufresne et al. 2005) (Fig. 20.10). The latter is likely due to the absence of genes encoding specific mismatch repair enzymes particularly those involved in the repair of G:C to A:T transversions (Rocap et al. 2003; Dufresne et al. 2005; Partensky and Garczarek 2010). Loss of such enzymes may have been due to the absence of UV radiation in the low light niche relaxing selection on the corresponding genes that thus became dispensable (Partensky and Garczarek 2010). This genome streamlining and bias towards an A+T-rich genome makes evolutionary sense in the context of the reduced nutrient requirements that such modifications impart, a smaller genome requiring les nitrogen and phosphorus whilst an AT base pair contains seven atoms of nitrogen compared to eight for a GC linkage (Hess 2008). Clearly, genome reduction cannot proceed below a certain limit, and for *Prochlorococcus* presumably lies close to the 1.64 Mb currently observed (Kettler et al. 2007) corresponding to a gene pool containing all the essential genes of biosynthetic pathways and housekeeping functions (so-called core genes, comprising 1,273 genes (Kettler et al. 2007) or 1,157 genes (Ting et al. 2009) in *Prochlorococcus* cf 1,572 core genes in marine *Synechococcus* (Dufresne et al. 2008)) plus a number of niche-specific genes and others that are unique to a particular strain (together comprising the accessory genome).

Despite a significant reduction in genome size in most *Prochlorococcus* lineages, important gene gains are also evident, likely a result of horizontal gene transfer from phage (Rocap et al. 2003; Coleman et al. 2006). At least in *Synechococcus* genomes, however, such gene gains may also be mediated via plasmids (Palenik et al. 2009). Such processes are likely responsible for the high diversity of gene complement in marine cyanobacterial genomes which at the population level is manifest in a *pan*-genome (i.e. the global gene repertoire of a taxon) that is several orders of magnitude greater than the genome of any single strain. Indeed,

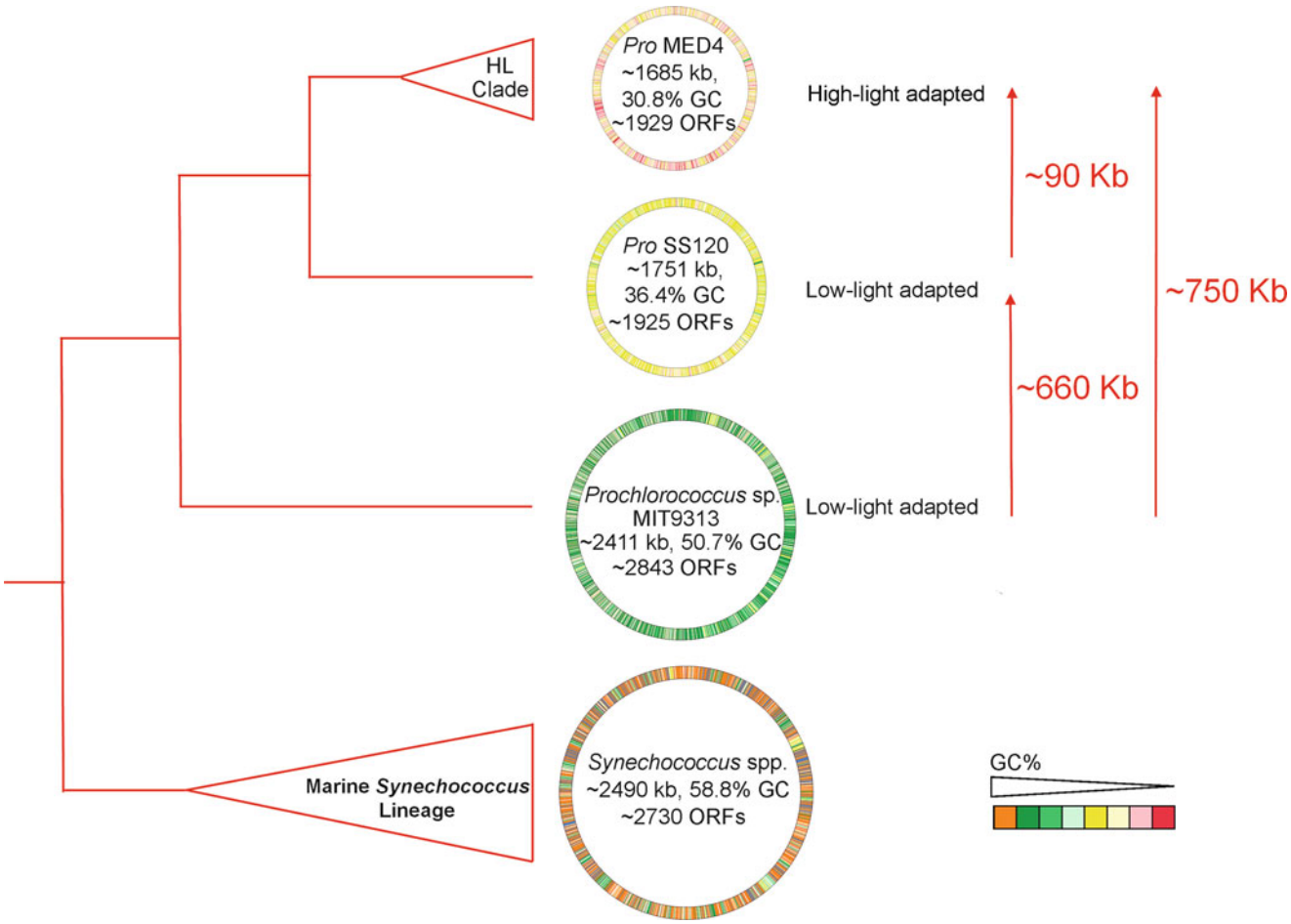

Fig. 20.10 Schematic representation of the genome streamlining process in *Prochlorococcus* (Image courtesy of Dr A. Dufresne)

mathematical modelling suggests that for every newly sequenced *Synechococcus* or *Prochlorococcus* genome the average discovery rate of new gene clusters is ~277, indicating an 'open' *pan*-genome that is very large and theoretically unlimited (Baumdicker et al. 2010). Such a gene pool would allow tremendous combinatorial flexibility in the accessory genome of specific strains or ecotypes. A mechanistic basis for such flexibility is seen in the clustering of unique genes into genomic islands (Fig. 20.11), suggesting these are hotspots for recombination having arisen via lateral gene transfer and are continually undergoing rearrangement (Coleman et al. 2006). Moreover, the fact that metagenomic sequence reads recruit relatively poorly to these island regions compared to high recruitment in conserved regions of the genome, reiterates the idea that a considerable pool of genes is accessible to natural *Prochlorococcus* and *Synechococcus* populations.

Whilst many of the genes contained within genomic islands are currently of unknown function, others known to be involved in cell surface modification (Sect. 20.8) the response to physiological stress, photosynthesis or nutrient uptake (Coleman et al. 2006; Dufresne et al. 2008) likely provide a clear selective advantage to the organisms in which they reside (Sect. 20.10). Moreover, there is evidence both of a large variation in island gene content, even between closely related strains, consistent with them providing a selective advantage to local environmental conditions within a specific ecotype or clade, as well as of island genes that are present in various *Prochlorococcus* or *Synechococcus* strains (Scanlan et al. 2009). The latter likely assist in the adaptation to larger spatial scales over evolutionary time periods and include the phycobilisome rod gene region which contains a variable number of genes and is predicted to be an island in all *Synechococcus* strains except those with the most primitive pigment types (types 1 and 2) (Six et al. 2007; Dufresne et al. 2008). The fact that such a region can be laterally transferred between *Synechococcus* strains points to an underlying mechanism for their accession of new light niches.

Phylogenetic analysis of concatenated core proteins derived from the different *Synechococcus* genomes has provided evidence for two discrete sub-groups within marine sub-cluster 5.1 that may have ecological relevance (Dufresne

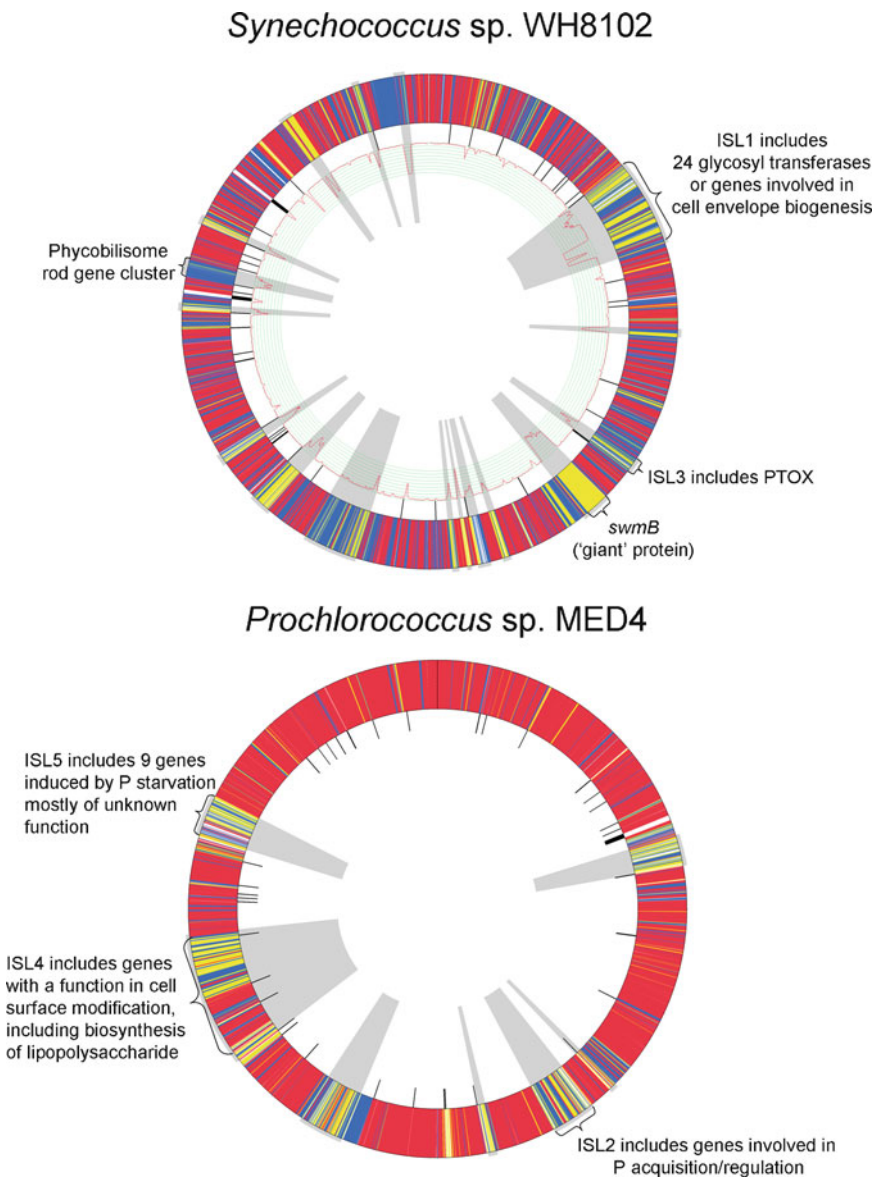

Fig. 20.11 Genome plot of recently acquired genomic islands in marine *Synechococcus* sp. WH8102 and *Prochlorococcus* sp. MED4. The outer circle represents individual genes: *red* = a core gene; *blue* = an accessory gene and *yellow* = a unique gene. The *shaded grey* regions represent genomic islands. For *Synechococcus* sp. WH8102 genomic islands were predicted based on deviation in tetranucleotide frequency greater than 1 standard deviation in the 1st principal component (i.e. the *red line* on the green scale) as compared to the genome average (see Dufresne et al. 2008). For *Prochlorococcus* sp. MED4 islands are largely those identified by Coleman et al. (2006) using breaks in synteny compared to closely related *Prochlorococcus* strains. The *black lines* inside the first circle are tRNAs, which can assist in determining the borders of individual islands. Some examples of specific island genes are indicated (Image courtesy of Dr A. Millard)

et al. 2008). Sub-group A comprises strains WH8102 (clade III), CC9605 (clade II), CC9902 and BL107 (both clade IV) whilst sub-group B includes WH7803 (clade V), WH7805 (clade VI), CC9311 (clade I), RS9916 (clade IX) and RS9917 (clade VIII). Such a division is also supported by phylogenetic networks based on shared gene content i.e. the phyletic distribution of accessory genes shared between the various *Synechococcus* genomes. These proposed sub-groups to some extent correspond to ecologically coherent groups being either open ocean/specialists that dominate in tropical/sub-tropical-oligotrophic or temperate/polar-mesotrophic waters (sub-group A) or coastal/opportunists that can be found either in coastal areas or across a broad range of habitats in relatively low numbers, but occasionally reaching higher numbers in the vicinity of upwelling areas or following environmental perturbation (sub-group B) (Dufresne et al. 2008). Given that broad ecological lifestyle signatures can be identified in marine picocyanobacterial genomes, there is thus a future potential that comparison of the 'population' genomes of these organisms at scales relevant to

specific ocean provinces for example, can provide important clues into the evolutionary pressures being imparted on specific components of each taxon. Indeed, analysis of *Prochlorococcus* metagenomic and genomic datasets has already provided evidence for local selection and enrichment of P metabolism genes in waters of low P availability (Martiny et al. 2006, 2009c; Rusch et al. 2007; Coleman and Chisholm 2010; Sect. 20.10) whilst biogeography of the light-harvesting *isiA* gene is consistent with its proposed role in enabling marine *Synechococcus* to acclimate to conditions of iron limitation (Bibby et al. 2009).

Marine picocyanobacterial genomes have a low density of genes encoding regulatory proteins, a feature particularly evident in those *Prochlorococcus* genomes subject to genome streamlining (Dufresne et al. 2003). This has been linked to the 'stable' environment occupied by specific *Prochlorococcus* ecotypes (Dufresne et al. 2003) a proposal also consistent with the higher number of two-component regulatory systems in sub-cluster B *Synechococcus* which have to cope with more variable environments than their sub-cluster A counterparts (Dufresne et al. 2008). However, the ocean environment clearly does fluctuate even over small spatial scales (Blackburn et al. 1998), so how might these organisms respond to such changing conditions? The answer appears to lie in the presence of non-coding RNAs (ncRNAs) which occur at levels typical of other bacteria, suggesting that they may have a disproportional regulatory role, at least in *Prochlorococcus* (Axmann et al. 2005; Steglich et al. 2008). In *Prochlorococcus* sp. MED4 some of these ncRNAs are differentially expressed under light stress adaptation and/or the response to phage infection, and a high number of them are found to be associated with genomic islands, suggesting functional links between these ncRNAs and the response of *Prochlorococcus* to particular environmental challenges and thus that the function of these molecules is relevant for determining the relative fitness of *Prochlorococcus* ecotypes (Steglich et al. 2008). Further weight for such an idea is given by the large number of ncRNAs that were found in meta-transcriptome datasets obtained from a natural microbial community at station ALOHA off Hawaii (Shi et al. 2009) i.e. that they are actually expressed *in situ*. Some of the ncRNAs identified could be specifically attributed to *Prochlorococcus*, including two experimentally validated ncRNAs (Yfr8 and Yfr9) which were found antisense to one another and were hypothesized to be involved in a toxin–antitoxin system in *Prochlorococcus* sp. MED4, systems which can be beneficial for cell survival under unfavourable growth conditions (Steglich et al. 2008). Moreover, a few *Prochlorococcus*-like ncRNA groups identified in the ALOHA datasets mapped to some but not all co-existing members of the *Prochlorococcus* community, suggesting that such ncRNAs may provide niche-specific regulation (Shi et al. 2009). For example, one group of putative environmental ncRNAs mapped only to the genome of *Prochlorococcus* sp. MIT9215 which in that strain are located in a hyper-variable region adjacent to phosphate transporter genes, and share a 14-bp exact match with the 5′ translation initiation site of the phosphate ABC transporter gene (*pstC*). This, and some other *Prochlorococcus* strains (Sect. 20.10) lack the *phoBR* two-component system (Scanlan and West 2002; Martiny et al. 2006), and it has been proposed that this group of ncRNAs might represent an alternative mechanism for regulating P assimilation in these organisms (Shi et al. 2009). Overall, the relatively high number of ncRNAs found in these organisms, and particularly *Prochlorococcus*, is intriguing and may represent a mode of adaptation to the extremely low nutrient conditions of the open oceans, regulation by ncRNAs requiring fewer resources than would be required for the synthesis of protein regulators.

20.10 Biological Adaptation to Light and Nutrients: The Niche Adaptation Paradigm

Whilst for marine *Synechococcus* the relationship between niche and genetic potential (i.e. the composition or regulation of gene complement) are still relatively poorly defined, for *Prochlorococcus* there is now excellent physiological and genomic data for defining the molecular basis of niche adaptation mechanisms particularly with respect to light and vertical partitioning down the water column (Moore et al. 1998; Rocap et al. 2003; Kettler et al. 2007). Light is obviously a critical parameter for a photosynthetic microbe like *Prochlorococcus* with the diel light-dark cycle clearly impacting on photosynthetic physiology (Claustre et al. 2002; Bruyant et al. 2005) and orchestrating gene expression (Holtzendorff et al. 2001; Zinser et al. 2009) even in the absence of a fully functioning (Holtzendorff et al. 2008), or at least simplified (Axmann et al. 2009), circadian clock.

Before focusing solely on the *Prochlorococcus* genus, let us consider more generally how fundamental differences in the light harvesting apparatus might dictate vertical distributions of the two genera since (Sect. 20.4) *Prochlorococcus* populations generally extend further down the photic zone than *Synechococcus* (Olson et al. 1990b; Partensky et al. 1999b; Johnson et al. 1999; Goericke et al. 2000), with members of the latter genus often disappearing below 150 m. A key facet is that the presence of a membrane-integral Pcb antenna can provide large absorption cross sections to both photosystems, and coupled to the small size of *Prochlorococcus*, means that the blue wavelengths which are enriched in deep waters have a greater chance of being absorbed rather than scattered (Ting et al. 2002). Indeed, in *Prochlorococcus* cells, the average ratio of energy absorbed

to energy striking its geometrical cross-section is almost twice that of *Synechococcus* (Morel et al. 1993). On the other hand, the phycobilisome antenna of *Synechococcus* is membrane extrinsic so no appression of the thylakoid is possible and hence the overall absorption cross-section of these strains is limited. Furthermore, the length of the phycobilisome rods is restricted by the need for lateral diffusion of the phycobilisome. The employment of a phycobilisome is therefore not conducive for optimal growth at depth.

The phycobilisome antenna of *Synechococcus* does, however, allow for large state transitions which serve to re-address imbalances in electron transport by selectively channelling excitation energy to either photosystem I (PSI) or photosystem II (PSII). This light-harvesting strategy has clear advantages for growth in the changing light environments of the surface mixed layers of the ocean. This information sits on top of strategies of marine *Synechococcus* to cope with excess light energy absorbed by the phycobilisomes, which include photoprotective energy dissipation mechanisms involving the orange carotenoid protein (Wilson et al. 2006, 2007), or means of alternative electron flow to oxygen, prior to PSI, where electrons are removed from the intersystem photosynthetic electron transport chain by an oxidase, possibly plastoquinol terminal oxidase (Bailey et al. 2008; Mackey et al. 2008; Grossman et al. 2010). In contrast, *Prochlorococcus* possesses a different type of non-photochemical quenching (NPQf) response (Bailey et al. 2005) and with the capacity for NPQf during increasing irradiance greater for the HL-adapted *Prochlorococcus* strain PCC9511 than the LL-adapted *Prochlorococcus* sp. SS120. This is consistent with the former strain being able to efficiently manage the fluctuating light intensities of the mixed layer whilst the lower capacity for NPQf, which saturates at lower irradiances, for *Prochlorococcus* sp. SS120 is coherent with its partitioning at lower depths, where light attenuation is greater and slower mixing ensures smaller changes in irradiance.

The underlying physiological diversity among *Prochlorococcus* strains that gives rise to the HL and LL-adapted classification arises from the ability of each strain to photoacclimate to a narrow range of light intensities. A number of laboratory and field studies have demonstrated that HL-adapted strains have higher optimal light intensities for growth when compared with LL strains (Moore et al. 1995, 1998; Moore and Chisholm 1999). Higher growth rates are also supported by higher levels of whole chain photosynthetic electron transport. In addition, HL and LL-adapted strains have very different pigment compositions with LL strains having a higher ratio of divinyl chlorophyll b_2/a_2 than their HL counterparts. The distinction between HL and LL ecotypes is further revealed by whole-genome comparisons (Rocap et al. 2003; Dufresne et al. 2003; Kettler et al. 2007). Thus, the HLI strain *Prochlorococcus* sp. MED4 possesses only one gene encoding the light-harvesting antenna protein Pcb (though most other HL-adapted strains have two copies, one for each photosystem), whereas the LLI strains NATL1A/NATL2A have seven (six PSII-associated and one PSI-associated), and the LLII strain SS120 has eight (six PSII-associated and two PSI-associated) (Garczarek et al. 2007). This latter strain is capable of growth at very low light intensities (Moore and Chisholm 1999). Interestingly, this multiplication of PSII-associated pcb genes did not occur in the 'primitive' LL lineage containing *Prochlorococcus* sp. MIT9313. Moreover, not including core genes HL-adapted *Prochlorococcus* isolates also share an additional ca 250 accessory genes, 95 of which are not found in any of the LL-adapted isolates (Kettler et al. 2007). All HL isolates (except *Prochlorococcus* sp. MED4) possess two photolyases known to be involved in the repair of cyclobutane pyrimidine dimers created by UV light. In contrast, *Prochlorococcus* sp. MED4 (Osburne et al. 2010) and members of the LLI ecotype possess one photolyase whilst all other currently sequenced LL strains lack the enzyme (Scanlan et al. 2009). Several other ecotypic differences in gene content involved in the response to DNA replication, recombination and repair have also been reported (Partensky and Garczarek 2010). The presence of a photolyase-like gene in the LLI ecotype is consistent with members of this ecotype being found even in high-latitude surface waters where high light stress and UV irradiation are prevalent (Johnson et al. 2006; Zinser et al. 2007; Malmstrom et al. 2010). Moreover, occupation of such waters also explains the large number (41) of high light-inducible protein (HLIP) genes (Kettler et al. 2007) found in this ecotype, many more than found in other LL strains (e.g. six in *Prochlorococcus* sp. SS120 and nine in *Prochlorococcus* sp. MIT9313), though why this number is significantly more than in HL strains themselves (e.g. 22 in *Prochlorococcus* sp. MED4 (Bhaya et al. 2002) and 24 in *Prochlorococcus* sp. MIT9312 (Coleman et al. 2006)) is unclear. In addition, the HL core contains dozens of conserved hypothetical genes not found in any LL isolate, and these genes might be critical for survival in the nutrient-poor, HL environment of the surface ocean (Kettler et al. 2007). Similarly, LL isolates share over 90 genes beyond the *Prochlorococcus* core, nearly 50 of which are not found in any HL isolates (Kettler et al. 2007). The latter includes several phycoerythrin genes (*cpeABSTYZ*) whereas HL isolates have lost all but *cpeB* and *cpeS* (Hess et al. 2001).

Although there are clear differences in gene content between HL- and LL–adapted isolates, within the two currently known HL-adapted ecotypes there is little that might explain the clear ecological preferences of the two lineages. However, given that it is temperature variability that is most strongly correlated with differences in abundance of the two ecotypes this could occur through more subtle changes to orthologous proteins that might yield different temperature optima for maximal activity or there may be more

genome-wide differences that affect membrane lipid composition alterations (Kettler et al. 2007).

In contrast, adaptation to nutrient stress, at least by modification of the spectrum of nutrient sources that can be accessed by a particular organism, may only require the acquisition of a few key genes (e.g., nitrite reductase and alkaline phosphatase). It follows then that the distribution of known nutrient genes in marine picocyanobacterial genomes and metagenomic datasets might correlate with the local availability of a particular nutrient (see Martiny et al. 2006, 2009b, c; Rusch et al. 2007; Coleman and Chisholm 2010). Indeed, of known *Prochlorococcus* genomes, strain MED4 containing the largest set of P-responsive genes (Martiny et al. 2006) originates from surface waters of the northwest Mediterranean Sea, where the P concentration is typically 100 nM and where P limitation of bacterioplankton has been documented (Zohary and Robarts 1998). Similarly, *Prochlorococcus* spp. NATL1A and NATL2A, which possess orthologs to most of the MED4 P-regulon region genes, came from surface waters in the central North Atlantic Ocean, where surface P levels were between 50 and 150 nM at the time these strains were isolated. Conversely, *Prochlorococcus* strains with the fewest orthologs to the P regulatory region were isolated from ocean regions with surface P concentrations >600 nM (Martiny et al. 2006; Coleman and Chisholm 2010). Such work has been reinforced by analysis of metagenomic datasets in which P acquisition genes surrounding the *pho*B regulator were found to be more prevalent in the Sargasso and Caribbean Sea compared with the Indian and Pacific Ocean, consistent with the low P concentrations in the respective environments at the time of sampling (Martiny et al. 2009c; Coleman and Chisholm 2010). Interestingly, another P-responsive region of the *Prochlorococcus* sp. MED4 genome (spanning genes located between PMM1403-PMM1419, though the genes are largely of unknown function), and commonly found among cells in low-P areas but not in high-P areas (Martiny et al. 2006), is found in a genomic island, ISL5, unique to this strain (Coleman et al. 2006) (Fig. 20.11). This mirrors the situation found in two other *Prochlorococcus* strains (MIT9301 and MIT9303) that harbour newly described genes (*phnYZ*) involved in 2-aminoethylphosphonate utilisation, homologues of which are significantly enriched in Sargasso Sea metagenomic datasets compared to those from station ALOHA in the Pacific, and which are also located within a genomic island (Martinez et al. 2010; Coleman and Chisholm 2010). Such results are consistent with phosphonate utilisation (and whatever component of the P-starvation response genes PMM1403-PMM1419 are involved in) being potentially recently acquired by horizontal gene transfer in these strains and providing a rapid selective advantage for *Prochlorococcus* in low DIP ecosystems.

Similar to phosphate limitation, it appears that genomic islands also play a role in adaptation to nitrogen limitation in *Prochlorococcus*. Thus, recent evidence suggests the widespread occurrence of a genomic island containing nitrite and nitrate assimilation genes in uncultured *Prochlorococcus* cells from marine surface waters (Martiny et al. 2009b). Moreover, these genes were found to be more prevalent in cells derived from the Caribbean Sea and Indian Ocean, regions characterized by low nitrogen at the time of sampling, than in those from the Sargasso Sea and Eastern Pacific, where nitrate concentrations are generally higher or cells are limited by other nutrients e.g., phosphate (Martiny et al. 2009b).

Gene content surveys for Fe stress genes have also shown considerable variation in sequenced marine picocyanobacterial genomes. This has revealed Fe related gene distributions in *Prochlorococcus* to more closely resemble those of coastal rather than open ocean *Synechococcus* genomes with every sequenced *Prochlorococcus* strain – isolated from a known Fe-limited region or not – containing Fe storage and substitution genes (Rivers et al. 2008) as well as a putative Fe regulator of the CRP family (Scanlan et al. 2009). Given the caveat that none of the so far sequenced open ocean *Synechococcus* strains are from regions of predicted Fe limitation (Boyd et al. 2007) the implication is that there is a greater selective pressure on *Synechococcus*, and particularly clades I and IV, to recruit Fe-responsive genes in or near coastal waters. However, whether this is due to the greater Fe requirement of members of these clades or to a more variable Fe supply is, as yet, unclear. Certainly, coastal Fe limitation has been observed, in areas including the California Current and the Peru Upwelling (Hutchins et al. 1998, 2002; Bruland et al. 2001). Noteworthy is that in the mesotroph/specialist strains *Synechococcus* sp. BL107 and CC9902 various putative Fe-responsive genes (e.g. those encoding ferritin, flavodoxin or Fe transport proteins) are located in a genomic island (Scanlan et al. 2009), whilst in the coastal/opportunist strain *Synechococcus* sp. CC9311 the same genes are not in an island as such, but rather have become 'fixed' in the accessory (i.e. non-core) genome suggesting a more ancient acquisition. Such differences clearly highlight how environmental selection can impact on the composition of marine picoyanobacterial genomes across varying timescales. Interestingly, consensus genomes for the *Prochlorococcus* HNLC lineages (section 20.7), whilst possessing a Fe acquisition/storage gene toolbox similar to other *Prochlorococcus* ecotypes, show a significant reduction in Fe-containing proteins (Rusch et al. 2010) suggesting a novel way for these organisms to reduce their Fe quota, further adding to our understanding of how marine phytoplankton adapt to variations in nutrient availability in the ocean.

Moving on from Fe, a specific comparison of the *Synechococcus* spp. CC9311 and WH8102 genomes, potentially representing different ends of the coastal-mesotroph:

open-ocean-oligotroph ecological strategy spectrum, has accentuated the view that the former strain has a metal-intensive ecological strategy, indicated by the larger number of genes involved in metal homeostasis as well as metal enzymes and sensor kinases that are not present in the oligotrophic strain (Palenik et al. 2006). Subsequent microarray experiments using the same strains (Stuart et al. 2009) and comparing the transcriptional response to copper shock, revealed that whilst the open-ocean strain showed a general stress response, the coastal strain exhibited a more specific oxidative or heavy-metal acclimation response that may confer copper tolerance. In addition, the coastal strain activated more regulatory elements and transporters, many of which are not conserved in other marine *Synechococcus* strains and may have been acquired by horizontal gene transfer (Stuart et al. 2009). Whilst further work is required to clarify whether these newly acquired genes do indeed contribute to copper tolerance, if this is so then this would be further evidence that *Synechococcus* species can adapt to a specific (micro)environment by acquiring and using novel genes when necessary.

20.11 Future Perspectives

Even with the relatively small number of picocyanobacterial genomes currently available, our knowledge of the molecular mechanisms used by these abundant photoautotrophs to occupy a specific niche has clearly moved forward significantly in recent years. Further rapid progress is envisaged particularly via accessing the 'population genome' of these organisms using a combination of metagenomic (Venter et al. 2004; Palenik et al. 2009; Coleman and Chisholm 2010; Rusch et al. 2010), transcriptomic (Frias-Lopez et al. 2008) and flow cytometry (Rodrigue et al. 2009) approaches. Given similar advances in the bioinformatic analysis of such datasets (e.g. see Gianoulis et al. 2009) important new insights into the selective pressures being endured in a particular environment by specific picocyanobacterial populations might be inferred.

Nonetheless there are still some hurdles to overcome. The *pan*-genome of these organisms clearly contributes thousands of new genes that will require annotation and/or functional characterisation. Although a genetic system is available for *Synechococcus* (Brahamsha 1996b), for *Prochlorococcus* only a single strain has thus far been manipulated, and at relatively inefficient rates (Tolonen et al. 2006a). Advances in improving *Prochlorococcus* plating efficiencies using 'helper' heterotrophic organisms will clearly be useful in this respect (Morris et al. 2008). Alternatively, further use of large-scale gene expression approaches should be adopted, particularly encompassing RNA-seq technologies, given the valuable information that has already been amassed using microarrays on the response to nutrient limitation/toxicity (Martiny et al. 2006; Tolonen et al. 2006b; Stuart et al. 2009; Tetu et al. 2009; Thomas et al. 2009) light quality and rhythmicity (Steglich et al. 2006; Zinser et al. 2009) and picocyanobacteria-heterotrophic bacteria interactions (Tai et al. 2009). Coupled with further computational prediction of regulatory networks (e.g. see Su et al. 2003, 2005, 2006, 2007; Mao et al. 2010) and characterisation of new regulatory molecules (Axmann et al. 2005; Steglich et al. 2008) we may soon be able to model the complete regulatory and metabolic network of a picocyanobacterial cell with high resolution. Indeed, integration of such molecular models into the framework of existing ecophysiological or ecosystem models (Follows et al. 2007; Rabouille et al. 2007; Bragg et al. 2010) has the potential to make great strides in our predictive understanding of the responses of these organisms to future climate change. There remains though much to learn about various aspects of picocyanobacterial biology, and particular their interaction with other organisms, be they be grazers, viruses or indeed mutually beneficial 'partners'. However, given the progress that has already been made, obtaining a complete understanding of the composition and workings of the marine microbial niche from the micro-scale to that of provinces or biomes is perhaps not such an unrealistic proposition for these important and abundant marine photoautotrophs.

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References

Ahlgren NA, Rocap G (2006) Culture isolation and culture-independent clone libraries reveal new marine *Synechococcus* ecotypes with distinctive light and N physiologies. Appl Environ Microbiol 72:7193–7204

Ahlgren NA, Rocap G, Chisholm SW (2006) Measurement of *Prochlorococcus* ecotypes using real-time polymerase chain reaction reveals different abundances of genotypes with similar light physiologies. Environ Microbiol 8:441–454

Andersson A, Haecky P, Hagström A (1994) Effect of temperature and light on the growth of micro- nano- and pico-plankton: impact on algal succession. Mar Biol 120:511–520

Apple JK, Strom SL, Palenik B, Brahamsha B (2011) Variability in protist grazing and growth on different marine *Synechococcus* isolates. Appl Environ Microbiol 77:3074–3084

Axmann IM, Kensche P, Vogel J, Kohl S, Herzel H, Hess WR (2005) Identification of cyanobacterial non-coding RNAs by comparative genome analysis. Genome Biol 6:R73

Axmann IM, Dühring U, Seeliger L, Arnold A, Vanselow JT, Kramer A, Wilde A (2009) Biochemical evidence for a timing mechanism in *Prochlorococcus*. J Bacteriol 191:5342–5347

Bailey S, Mann NH, Robinson C, Scanlan DJ (2005) The occurrence of rapidly reversible non-photochemical quenching of chlorophyll *a* fluorescence in cyanobacteria. FEBS Lett 579:275–280

Bailey S, Melis A, Mackey KR, Cardol P, Finazzi G, van Dijken G, Berg GM, Arrigo K, Shrager J, Grossman A (2008) Alternative photosynthetic electron flow to oxygen in marine *Synechococcus*. Biochim Biophys Acta 1777:269–276

Baudoux AC, Veldhuis MJW, Witte HJ, Brussaard CPD (2007) Viruses as mortality agents of picophytoplankton in the deep chlorophyll maximum layer during IRONAGES III. Limnol Oceanogr 52:2519–2529

Baudoux AC, Veldhuis MJW, Noordeloos AAM, van Noort G, Brussaard CPD (2008) Estimates of virus- vs. grazing induced mortality of picophytoplankton in the North Sea during summer. Aquat Microb Ecol 52:69–82

Baumdicker F, Hess WR, Pfaffelhuber P (2010) The diversity of a distributed genome in bacterial populations. Annal Appl Prob 20:1567–1606

Bertilsson S, Berglund O, Karl DM, Chisholm SW (2003) Elemental composition of marine *Prochlorococcus* and *Synechococcus*: implications for the ecological stoichiometry of the sea. Limnol Oceanogr 48:1721–1731

Bhaya D, Dufresne A, Vaulot D, Grossman A (2002) Analysis of the *hli* gene family in marine and freshwater cyanobacteria. FEMS Microbiol Lett 215:209–219

Bibby TS, Mary I, Nield J, Partensky F, Barber J (2003) Low-light-adapted *Prochlorococcus* species possess specific antennae for each photosystem. Nature 424:1051–1054

Bibby TS, Zhang Y, Chen M (2009) Biogeography of marine light-harvesting systems in marine phytoplankton. PLoS One 4:e4601

Blackburn N, Fenchel T, Mitchell J (1998) Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. Science 282:2254–2256

Blanchot J, Rodier M (1996) Phytoplankton abundance and biomass in the western tropical Pacific Ocean during the 1992 El Niño year: new data from flow cytometry. Deep Sea Res Part I 43:877–895

Blot N, Wu XJ, Thomas J-C, Zhang J, Garczarek L, Böhm S, Tu JM, Zhou M, Plöscher M, Eichacker L, Partensky F, Scheer H, Zhao KH (2009) Phycourobilin in trichromatic phycocyanin from oceanic cyanobacteria is formed post-translationally by a phycoerythrobilin lyase-isomerase. J Biol Chem 284:9290–9298

Boenigk J, Matz C, Jürgens K, Arndt H (2001) The influence of preculture conditions and food quality on the ingestion and digestion process of three species of heterotrophic nanoflagellates. Microb Ecol 42:168–176

Bopp L, Aumont O, Cadule P, Alvain S, Gehlen M (2005) Response of diatoms distribution to global warming and potential implications: a global model study. Geophys Res Lett 32:L19606

Bouman HA, Ulloa O, Scanlan DJ, Zwirglmaier K, Li WKW, Platt T, Stuart V, Barlow R, Leth O, Clementson L, Lutz V, Fukasawa M, Watanabe S, Sathyendranath S (2006) Oceanographic basis of the global surface distribution of *Prochlorococcus* ecotypes. Science 312:918–921

Bouman HA, Ulloa O, Barlow R, Li KW, Platt T, Zwirglmaier K, Scanlan DJ, Sathyendranath S (2011) Water-column stratification governs the community structure of subtropical marine picophytoplankton. Environ Microbiol Rep 3:473–482

Boyd PW, Jickells T, Law CS, Blain S, Boyle EA, Buesseler KO, Coale KH, Cullen JJ, de Baar HJW, Follows M, Harvey M, Lancelot C, Levasseur M, Owens NPJ, Pollard R, Rivkin RB, Sarmiento J, Schoemann V, Smetacek V, Takeda S, Tsuda A, Turner S, Watson AJ (2007) Mesoscale iron enrichment experiments 1993–2005: synthesis and future directions. Science 315:612–617

Bragg JG, Dutkiewicz S, Jahn O, Follows MJ, Chisholm SW (2010) Modeling selective pressures on phytoplankton in the global ocean. PLoS One 5:e9569

Brahamsha B (1996a) An abundant cell-surface polypeptide is required for swimming by the nonflagellated marine cyanobacterium *Synechococcus*. Proc Natl Acad Sci USA 93:6504–6509

Brahamsha B (1996b) A genetic manipulation system for oceanic cyanobacteria of the genus *Synechococcus*. Appl Environ Microbiol 62:1747–1751

Brahamsha B (1999) Non-flagellar swimming in marine *Synechococcus*. J Mol Microbiol Biotechnol 1:59–62

Brown MV, Fuhrman JA (2005) Marine bacterial microdiversity as revealed by internal transcribed spacer analysis. Aquat Microb Ecol 41:15–23

Brown MV, Schwalbach MS, Hewson I, Fuhrman JA (2005) Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity: development and application to a time series. Environ Microbiol 7:1466–1479

Bruland KW, Rue EL, Smith GJ (2001) Iron and macronutrients in California coastal upwelling regimes: implications for diatom blooms. Limnol Oceanogr 46:1661–1674

Bruyant F, Babin M, Genty B, Prasil O, Behrenfeld MJ, Claustre H, Bricaud A, Garczarek L, Holtzendorff J, Koblizek M, Dousova H, Partensky F (2005) Diel variations in the photosynthetic parameters of *Prochlorococcus* strain PCC 9511: combined effects of light and cell cycle. Limnol Oceanogr 50:850–863

Buck KR, Chavez FP, Campbell L (1996) Basin-wide distributions of living carbon components and the inverted trophic pyramid of the central gyre of the North Atlantic Ocean, summer 1993. Aquat Microb Ecol 10:283–298

Buesseler KO, Lamborg CH, Boyd PW, Lam PJ, Trull TW, Bidigare RR, Bishop JKB, Casciotti KL, Dehairs F, Elskens M, Honda M, Karl DM, Siegel DA, Silver MW, Steinberg DK, Valdes J, Van Mooy B, Wilson S (2007) Revisiting carbon flux through the ocean's twilight zone. Science 316:567–570

Burkill PH, Leakey RJG, Owens NJP, Mantoura RFC (1993) *Synechococcus* and its importance to the microbial food web of the northwestern Indian Ocean. Deep Sea Res Part II 40:773–782

Campbell L, Iturriaga R (1988) Identification of *Synechococcus* spp. in the Sargasso Sea by immunofluorescence and fluorescence excitation spectroscopy performed on individual cells. Limnol Oceanogr 33:1196–1201

Campbell L, Vaulot D (1993) Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep Sea Res Part I 40:2043–2060

Campbell L, Nolla HA, Vaulot D (1994) The importance of *Prochlorococcus* to community structure in the central North Pacific. Limnol Oceanogr 39:954–961

Campbell L, Liu H, Nolla HA, Vaulot D (1997) Annual variability of phytoplankton and bacteria in the subtropical North Pacific Ocean at Station ALOHA during the 1991–1994 ENSO event. Deep Sea Res Part I 44:167–192

Caron DA, Lim EL, Miceli G, Waterbury JB, Valois FW (1991) Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. Mar Ecol Prog Ser 76:205–217

Carr NG, Mann NH (1994) The oceanic cyanobacterial picoplankton. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp 27–48, 881 pp

Casey JR, Lomas MW, Mandecki J, Walker DE (2007) *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. Geophys Res Lett 34:L10604

Charpy L, Blanchot J (1998) Photosynthetic picoplankton in French Polynesia atoll lagoon: estimation of taxa contribution to biomass and production by flow cytometry. Mar Ecol Prog Ser 162:57–70

Chen F, Wang K, Kan JJ, Suzuki MT, Wommack KE (2006) Diverse and unique picocyanobacteria in Chesapeake Bay, revealed by 16S-23S rRNA internal transcribed spacer sequences. Appl Environ Microbiol 72:2239–2243

Chisholm SW (1992) Phytoplankton size. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum Press, New York, pp 213–237

Chisholm SW, Olson RJ, Zettler ER, Waterbury J, Goericke R, Welschmeyer N (1988) A novel free-living prochlorophyte occurs at high cell concentrations in the oceanic euphotic zone. Nature 334:340–343

Chisholm SW, Frankel SL, Goericke R, Olson RJ, Palenik B, Waterbury JB, West-Johnsrud L, Zettler ER (1992) *Prochlorococcus marinus nov*. gen. *nov*. sp.: an oxyphototrophic marine prokaryote containing divinyl chlorophyll *a* and *b*. Arch Microbiol 157:297–300

Choi DH, Noh JH (2009) Phylogenetic diversity of *Synechococcus* strains isolated from the East China Sea and the East Sea. FEMS Microbiol Ecol 69:439–448

Christaki U, Jacquet S, Dolan JR, Vaulot D, Rassoulzadegan F (1999) Growth and grazing on *Prochlorococcus* and *Synechococcus* by two marine ciliates. Limnol Oceanogr 44:52–61

Christaki U, Courties C, Karayanni H, Giannakourou A, Maravelias C, Kormas KA, Lebaron P (2002) Dynamic characteristics of *Prochlorococcus* and *Synechococcus* consumption by bacterivorous nanoflagellates. Microb Ecol 43:341–352

Claustre H, Bricaud A, Babin M, Bruyant F, Guillou L, Le Gall F, Marie D, Partensky F (2002) Diel variations in *Prochlorococcus* optical properties. Limnol Oceanogr 47:1637–1647

Coleman ML, Chisholm SW (2007) Code and context: *Prochlorococcus* as a model for cross-scale biology. Trends Microbiol 15:398–407

Coleman ML, Chisholm SW (2010) Ecosystem-specific selection pressures revealed through comparative population genomics. Proc Natl Acad Sci USA 107:18634–18639

Coleman ML, Sullivan MB, Martiny AC, Steglich C, Barry K, DeLong EF, Chisholm SW (2006) Genomic islands and the ecology and evolution of *Prochlorococcus*. Science 311:1768–1770

Cottrell MT, Kirchman DL (2009) Photoheterotrophic microbes in the Arctic Ocean in summer and winter. Appl Environ Microbiol 75:4958–4966

Cottrell MT, Michelou VK, Nemcek N, DiTullio G, Kirchman DL (2008) Carbon cycling by microbes influenced by light in the Northeast Atlantic Ocean. Aquat Microb Ecol 50:239–250

Davey M, Tarran GA, Mills MM, Ridame C, Geider RJ, LaRoche J (2008) Nutrient limitation of picophytoplankton photosynthesis and growth in the tropical North Atlantic. Limnol Oceanogr 53:1722–1733

Dufresne A, Salanoubat M, Partensky F, Artiguenave F, Axmann IM, Barbe V, Duprat S, Galperin MY, Koonin EV, Le Gall F, Makarova KS, Ostrowski M, Oztas S, Robert C, Rogozin IB, Scanlan DJ, Tandeau de Marsac N, Weissenbach J, Wincker P, Wolf YI, Hess WR (2003) Genome sequence of the cyanobacterium *Prochlorococcus marinus* SS120, a nearly minimal oxyphototrophic genome. Proc Natl Acad Sci USA 100:10020–10025

Dufresne A, Garczarek L, Partensky F (2005) Accelerated evolution associated with genome reduction in a free-living prokaryote. Genome Biol 6:R14

Dufresne A, Ostrowski M, Scanlan DJ, Garczarek L, Mazard S, Palenik BP, Paulsen IT, Tandeau de Marsac N, Wincker P, Dossat C, Ferriera S, Johnson J, Post AF, Hess WR, Partensky F (2008) Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. Genome Biol 9:R90

Durand MD, Olson RJ, Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda Atlantic time series station in the Sargasso Sea. Deep Sea Res Part II 48:1983–2003

Everroad C, Six C, Partensky F, Thomas JC, Holtzendorff J, Wood AM (2006) Biochemical bases of type IV chromatic adaptation in marine *Synechococcus* spp. J Bacteriol 188:3345–3356

Ferris MJ, Palenik B (1998) Niche adaptation in ocean cyanobacteria. Nature 396:226–228

Follows MJ, Dutkiewicz S, Grant S, Chisholm SW (2007) Emergent biogeography of microbial communities in a model ocean. Science 315:1843–1846

Fouilland E, Descolas-Gros C, Courties C, Pons V (1999) Autotrophic carbon assimilation and biomass from size-fractionated phytoplankton in the surface waters across the subtropical frontal zone (Indian Ocean). Polar Biol 21:90–96

Frias-Lopez J, Shi Y, Tyson GW, Coleman ML, Schuster SC, Chisholm SW, DeLong EF (2008) Microbial community gene expression in ocean surface waters. Proc Natl Acad Sci USA 105:3805–3810

Frias-Lopez J, Thompson A, Waldbauer J, Chisholm SW (2009) Use of stable isotope-labelled cells to identify active grazers of picocyanobacteria in ocean surface waters. Environ Microbiol 11:512–525

Fuller NJ, Marie D, Partensky F, Vaulot D, Post AF, Scanlan DJ (2003) Clade-specific 16S ribosomal DNA oligonucleotides reveal the predominance of a single marine *Synechococcus* clade throughout a stratified water column in the Red Sea. Appl Environ Microbiol 69:2430–2443

Fuller NJ, West NJ, Marie D, Yallop M, Rivlin T, Post AF, Scanlan DJ (2005) Dynamics of community structure and phosphate status of picocyanobacterial populations in the Gulf of Aqaba, Red Sea. Limnol Oceanogr 50:363–375

Fuller NJ, Tarran GA, Yallop M, Orcutt KM, Scanlan DJ (2006) Molecular analysis of picocyanobacterial community structure along an Arabian Sea transect reveals distinct spatial separation of lineages. Limnol Oceanogr 51:2515–2526

Furnas M (1991) Net in situ growth rates of phytoplankton in an oligotrophic, tropical shelf ecosystem. Limnol Oceanogr 36:13–29

Furnas M, Crosbie NJ (1999) In situ growth dynamics of the photosynthetic prokaryotic picoplankters *Synechococcus* and *Prochlorococcus*. Bull Inst Oceanogr 19:387–417

Garcia-Fernández JM, Tandeau de Marsac N, Diez J (2004) Streamlined regulation and gene loss as adaptive mechanisms in *Prochlorococcus* for optimized nitrogen utilization in oligotrophic environments. Microbiol Mol Biol Rev 68:630–638

Garczarek L, Hess WR, Holtzendorff J, van der Staay GW, Partensky F (2000) Multiplication of antenna genes as a major adaptation to low light in a marine prokaryote. Proc Natl Acad Sci USA 97:4098–4101

Garczarek L, van der Staay GWM, Hess WR, LeGall F, Partensky F (2001) Expression and phylogeny of the multiple antenna genes of the low-light-adapted strain *Prochlorococcus marinus* SS120 (Oxyphotobacteria). Plant Mol Biol 46:683–693

Garczarek L, Dufresne A, Rousvoal S, West NJ, Mazard S, Marie D, Claustre H, Raimbault P, Post AF, Scanlan DJ, Partensky F (2007) High vertical and low horizontal diversity of *Prochlorococcus* ecotypes in the Mediterranean Sea in summer. FEMS Microbiol Ecol 60:189–206

Garneau ME, Vincent WF, Alonso-Saez L, Gratton Y, Lovejoy C (2006) Prokaryotic community structure and heterotrophic production in a river-influenced coastal arctic ecosystem. Aquat Microb Ecol 42:27–40

Gianoulis TA, Raes J, Patel PV, Bjornson R, Korbel JO, Letunic I, Yamada T, Paccanaro A, Jensen LJ, Snyder M, Bork P, Gerstein MB (2009) Quantifying environmental adaptation of metabolic pathways in metagenomics. Proc Natl Acad Sci USA 106:1374–1379

Gieskes WWC, Kraay GW (1983) Unknown chlorophyll *a* derivatives in the North Sea and the tropical Atlantic Ocean revealed by HPLC analysis. Limnol Oceanogr 28:757–766

Glazer AN (1999) Cyanobacterial photosynthetic apparatus: an overview. Bull Inst Oceanogr 19:419–434

Glover HE (1985) The physiology and ecology of the marine cyanobacterial genus *Synechococcus*. Adv Aquat Microbiol 3:49–107

Goericke R, Repeta DJ (1992) The pigments of *Prochlorococcus marinus*: the presence of divinyl chlorophyll *a* and *b* in a marine prochlorophyte. Limnol Oceanogr 37:425–433

Goericke R, Welschmeyer NA (1993) The marine prochlorophyte *Prochlorococcus* contributes significantly to phytoplankton biomass

and primary production in the Sargasso Sea. Deep Sea Res Part I 40:2283–2294

Goericke R, Olson RJ, Shalapyonok A (2000) A novel niche for *Prochlorococcus* sp in low-light suboxic environments in the Arabian Sea and the Eastern Tropical North Pacific. Deep Sea Res Part I 47:1183–1205

Gradinger R, Lenz J (1989) Picocyanobacteria in the high Arctic. Mar Ecol Prog Ser 52:99–101

Gradinger R, Lenz J (1995) Seasonal occurrence of picocyanobacteria in the Greenland Sea and central Arctic Ocean. Polar Biol 15:447–452

Grossman AR, Mackey KRM, Bailey S (2010) A perspective on photosynthesis in the oligotrophic oceans: hypotheses concerning alternate routes of electron flow. J Phycol 56:629–634

Guillou L, Jacquet S, Chretiennot-Dinet MJ, Vaulot D (2001) Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. Aquat Microb Ecol 26:201–207

Haverkamp T, Acinas SG, Doeleman M, Stomp M, Huisman J, Stal LJ (2008) Diversity and phylogeny of Baltic Sea picocyanobacteria inferred from their ITS and phycobiliprotein operons. Environ Microbiol 10:174–188

Haverkamp THA, Schouten D, Doeleman M, Wollenzien U, Huisman J, Stal LJ (2009) Colourful microdiversity of *Synechococcus* strains (picocyanobacteria) isolated from the Baltic Sea. ISME J 3:397–408

Heldal M, Scanlan DJ, Norland S, Thingstad F, Mann NH (2003) Elemental composition of single cells of various strains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis. Limnol Oceanogr 48:1732–1743

Herdman M, Castenholz RW, Iteman I, Waterbury JB, Rippka R (2001) Subsection I (Formerly *Chroococcales* Wettstein 1924, emend. Rippka, Deruelles, Waterbury, Herdman and Stanier 1979). In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn, The archaea and the deeply branching and phototrophic bacteria. Springer, New York, pp 493–514

Hess WR (2004) Genome analysis of marine photosynthetic microbes and their global role. Curr Opin Biotechnol 15:191–198

Hess WR (2008) Comparative genomics of marine cyanobacteria and their phages. In: Herrero A, Flores E (eds) The cyanobacteria: molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 89–116, 484 pp

Hess WR, Partensky F, van der Staay GWM, Garcia-Fernández JM, Boerner T, Vaulot D (1996) Coexistence of phycoerythrin and a chlorophyll *a/b* antenna in a marine prokaryote. Proc Natl Acad Sci USA 93:11126–11130

Hess WR, Rocap G, Ting CS, Larimer F, Stilwagen S, Lamerdin J, Chisholm SW (2001) The photosynthetic apparatus of *Prochlorococcus*: insights through comparative genomics. Photosynth Res 70:53–71

Heywood JL, Zubkov MV, Tarran GA, Fuchs BM, Holligan PM (2006) Prokaryoplankton standing stocks in oligotrophic gyre and equatorial provinces of the Atlantic Ocean: evaluation of inter-annual variability. Deep Sea Res Part II 53:1530–1547

Hoiczyk E, Hansel A (2000) Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J Bacteriol 182:1191–1199

Holtzendorff J, Partensky F, Jacquet S, Bruyant F, Marie D, Garczarek L, Mary I, Vaulot D, Hess WR (2001) Diel expression of cell cycle-related genes in synchronized cultures of *Prochlorococcus* sp. strain PCC 9511. J Bacteriol 183:915–920

Holtzendorff J, Partensky F, Mella D, Lennon JF, Hess WR, Garczarek L (2008) Genome streamlining results in loss of robustness of the circadian clock in the marine cyanobacterium *Prochlorococcus marinus* PCC 9511. J Biol Rhythms 23:187–199

Honda D, Yokota A, Sugiyama J (1999) Detection of seven major evolutionary lineages in cyanobacteria based on 16S rRNA gene sequence analysis with new sequences of five marine *Synechococcus* strains. J Mol Evol 48:723–739

Huang S, Wilhelm SW, Harvey HR, Taylor K, Jiao N, Chen F (2012) Novel lineages of *Prochlorococcus* and *Synechococcus* in the global ocean. ISME J 6:285–297

Hutchins DA, DiTullio GR, Zhang Y, Bruland KW (1998) A Iron limitation mosaic in the California upwelling regime. Limnol Oceanogr 43:1037–1054

Hutchins DA, Hare CE, Weaver RS, Zhang Y, Firme GF, DiTullio GR, Alm MB, Riseman SF, Maucher JM, Geesey ME, Trick CG, Smith GJ, Rue EL, Conn J, Bruland KW (2002) Phytoplankton iron limitation in the Humboldt Current and Peru Upwelling. Limnol Oceanogr 47:997–1011

Jameson E, Joint I, Mann NH, Mühling M (2010) Detailed analysis of the microdiversity of *Prochlorococcus* populations along a north-south Atlantic Ocean transect. Environ Microbiol 12:156–171

Jardillier L, Zubkov MV, Pearman J, Scanlan DJ (2010) Significant CO_2 fixation by small prymnesiophytes in the subtropical and tropical northeast Atlantic Ocean. ISME J 4:1180–1192

Jenkins BD, Zehr JP, Gibson A, Campbell L (2006) Cyanobacterial assimilatory nitrate reductase gene diversity in coastal and oligotrophic marine environments. Environ Microbiol 8:2083–2095

Jezberová J, Komárková J (2007) Morphological transformation in a freshwater *Cyanobium* sp. induced by grazers. Environ Microbiol 9:1858–1862

Jing H, Liu H, Suzuki K (2009a) Phylogenetic diversity of marine *Synechococcus* spp. in the Sea of Okhotsk. Aquat Microb Ecol 56:55–63

Jing H, Zhang R, Pointing SB, Liu H, Qian P (2009b) Genetic diversity and temporal variation of the marine *Synechococcus* community in the subtropical coastal waters of Hong Kong. Can J Microbiol 55:311–318

Johnson PW, Sieburth JMcN (1979) Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. Limnol Oceanogr 24:928–935

Johnson Z, Landry ML, Bidigare RR, Brown SL, Campbell L, Gunderson J, Marra J, Trees C (1999) Energetics and growth kinetics of a deep *Prochlorococcus* spp. population in the Arabian Sea. Deep Sea Res Part II 46:1719–1743

Johnson ZI, Zinser ER, Coe A, McNulty NP, Woodward EM, Chisholm SW (2006) Niche partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients. Science 311:1737–1740

Jones H, Ostrowski M, Scanlan DJ (2006) A suppression subtractive hybridization approach reveals niche-specific genes that may be involved in predator avoidance in marine *Synechococcus* isolates. Appl Environ Microbiol 72:2730–2737

Joux F, Servais P, Naudin JJ, Lebaron P, Oriol L, Courties C (2005) Distribution of picophytoplankton and bacterioplankton along a river plume gradient in the Mediterranean Sea. Vie Milieu Life Environ 55:197–208

Karl DM, Lukas R (1996) The Hawaii Ocean Time-series (HOT) program: background, rationale and field implementation. Deep Sea Res Part II 43:129–156

Karl DM, Bidigare RR, Letelier RM (2001) Long-term changes in plankton community structure and productivity in the North Pacific Subtropical Gyre: the domain shift hypothesis. Deep Sea Res Part II 48:1449–1470

Kettler G, Martiny AC, Huang K, Zucker J, Coleman ML, Rodrigue S, Chen F, Lapidus A, Ferriera S, Johnson J, Steglich C, Church G, Richardson P, Chisholm SW (2007) Patterns and implications of gene gain and loss in the evolution of *Prochlorococcus*. PLoS Genet 3:e231

Kimmance SA, Wilson WH, Archer SD (2007) Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: limitations associated with method sensitivity in natural waters. Aquat Microb Ecol 49:207–222

Klähn S, Steglich C, Hess WR, Hagemann M (2010) Glucosylglycerate: a secondary compatible solute common to marine picocyanobacteria from nitrogen-poor environments. Environ Microbiol 12:83–94

Kuosa H (1991) Picoplanktonic algae in the northern Baltic Sea – seasonal dynamics and flagellate grazing. Mar Ecol Prog Ser 73:269–276

Kursar TA, Swift H, Alberte RS (1981) Morphology of a novel cyanobacterium and characterisation of light-harvesting complexes from it: implications for phycobiliprotein evolution. Proc Natl Acad Sci USA 78:6888–6892

LaRoche J, van der Staay GW, Partensky F, Ducret A, Aebersold R, Li R, Golden SS, Hiller RG, Wrench PM, Larkum AW, Green BR (1996) Independent evolution of the prochlorophyte and green plant chlorophyll *a/b* light-harvesting proteins. Proc Natl Acad Sci USA 93:15244–15248

Lavin P, Gonzáles B, Santibáñez JF, Scanlan DJ, Ulloa O (2010) Novel lineages of *Prochlorococcus* thrive within the oxygen minimum zone of the eastern tropical South Pacific. Environ Microbiol Rep 2:728–738

Li WKW (1994) Primary productivity of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: measurements from flow cytometric sorting. Limnol Oceanogr 39:169–175

Li WKW (1998) Annual average abundance of heterotrophic bacteria and *Synechococcus* in surface ocean waters. Limnol Oceanogr 43:1746–1753

Li WKW, Subba Rao DV, Harrison WG, Smith JC, Cullen JJ, Irwin B, Platt T (1983) Autotrophic picoplankton in the tropical ocean. Science 219:292–295

Lichtlé C, Thomas JC, Spilar A, Partensky F (1995) Immunological and ultrastructural characterisation of the photosynthetic complexes of the prochlorophyte *Prochlorococcus* (oxychlorobacteria). J Phycol 31:934–941

Lindell D, Post AF (1995) Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. Limnol Oceanogr 40:1130–1141

Lindell D, Padan E, Post AF (1998) Regulation of *ntcA* expression and nitrite uptake in the marine *Synechococcus* sp. strain WH 7803. J Bacteriol 180:1878–1886

Liu HB, Campbell L, Landry MR (1995) Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. Mar Ecol Prog Ser 116:277–287

Liu HB, Campbell L, Landry MR, Nolla HA, Brown SL, Constantinou J (1998) *Prochlorococcus* and *Synechococcus* growth rates and contributions to production in the Arabian Sea during the 1995 southwest and northeast monsoons. Deep Sea Res Part II 45:2327–2352

Mackey KRM, Paytan A, Grossman AR, Bailey S (2008) A photosynthetic strategy for coping in a high-light, low-nutrient environment. Limnol Oceanogr 53:900–913

Mackey KRM, Rivlin T, Grossman AR, Post AF, Paytan A (2009) Picophytoplankton responses to changing nutrient and light regimes during a bloom. Mar Biol 156:1531–1546

Malmstrom RR, Coe A, Kettler GC, Martiny AC, Frias-Lopez J, Zinser ER, Chisholm SW (2010) Temporal dynamics of *Prochlorococcus* ecotypes in the Atlantic and Pacific oceans. ISME J 4:1252–1264

Mann NH (2003) Phages of the marine cyanobacterial picophytoplankton. FEMS Microbiol Rev 27:17–34

Mann EL, Chisholm SW (2000) Iron limits the cell division rate of *Prochlorococcus* in the eastern equatorial Pacific. Limnol Oceanogr 45:1067–1076

Mann EL, Ahlgren N, Moffett JW, Chisholm SW (2002) Copper toxicity and cyanobacteria ecology in the Sargasso Sea. Limnol Oceanogr 47:976–988

Mao X, Olman V, Stuart R, Paulsen IT, Palenik B, Xu Y (2010) Computational prediction of the osmoregulation network in *Synechococcus* sp. WH8102. BMC Genomics 11:291

Marañón E, Holligan PM, Varela M, Mouriño B, Bale AJ (2000) Basin–scale variability of phytoplankton biomass, production and growth in the Atlantic Ocean. Deep Sea Res Part I 47:825–857

Marañón E, Holligan PM, Barciela R, Gonzalez N, Mourino B, Pazo MJ, Varela M (2001) Patterns of phytoplankton size structure and productivity in contrasting open-ocean environments. Mar Ecol Prog Ser 216:43–56

Marchant HJ, Davidson AT, Wright SW (1987) The distribution and abundance of chroococcoid cyanobacteria in the Southern Ocean. Proc NIPR Symp Polar Biol 1:1–9

Martin AP, Zubkov MV, Burkill PH, Holland RJ (2005) Extreme spatial variability in marine picoplankton and its consequences for interpreting Eulerian time-series. Biol Lett 1:366–369

Martin AP, Zubkov MV, Fasham MJ, Burkill PH, Holland RJ (2008) Microbial spatial variability: an example from the Celtic Sea. Prog Oceanogr 76:443–465

Martinez A, Tyson GW, DeLong EF (2010) Widespread known and novel phosphonate utilization pathways in marine bacteria revealed by functional screening and metagenomic analyses. Environ Microbiol 12:222–238

Martiny AC, Coleman ML, Chisholm SW (2006) Phosphate acquisition genes in *Prochlorococcus* ecotypes: evidence for genome-wide adaptation. Proc Natl Acad Sci USA 103:12552–12557

Martiny AC, Huang Y, Li W (2009a) Occurrence of phosphate acquisition genes in *Prochlorococcus* cells from different oceanic regions. Environ Microbiol 11:1340–1347

Martiny AC, Kathuria S, Berube PM (2009b) Widespread metabolic potential for nitrite and nitrate assimilation among *Prochlorococcus* ecotypes. Proc Natl Acad Sci USA 106:10787–10792

Martiny AC, Tai APK, Veneziano D, Primeau F, Chisholm SW (2009c) Taxonomic resolution, ecotypes and the biogeography of *Prochlorococcus*. Environ Microbiol 11:823–832

Mazard S, Ostrowski M, Partensky F, Scanlan DJ (2012) Multi-locus sequence analysis, taxonomic resolution and biogeography of marine *Synechococcus*. Environ Microbiol 14:372–386

McCarren J, Brahamsha B (2005) Transposon mutagenesis in a marine *Synechococcus* strain: isolation of swimming motility mutants. J Bacteriol 187:4457–4462

McCarren J, Brahamsha B (2007) SwmB, a 1.12-megadalton protein that is required for nonflagellar swimming motility in *Synechococcus*. J Bacteriol 189:1158–1162

McCarren J, Heuser J, Roth R, Yamada N, Martone M, Brahamsha B (2005) Inactivation of *swmA* results in the loss of an outer cell layer in a swimming *Synechococcus* strain. J Bacteriol 187:224–230

Mella-Flores D, Mazard S, Humily F, Partensky F, Mahé F, Bariat L, Courties C, Marie D, Ras J, Mauriac R, Jeanthon C, Mahdi-Bendif E, Ostrowski M, Scanlan DJ, Garczarek L (2011) Is the distribution of *Prochlorococcus* and *Synechococcus* ecotypes in the Mediterranean Sea affected by global warming? Biogeosciences 8:2785–2804

Moore LR, Chisholm SW (1999) Photophysiology of the marine cyanobacterium *Prochlorococcus*: ecotypic differences among cultured isolates. Limnol Oceanogr 44:628–638

Moore LR, Goericke R, Chisholm SW (1995) Comparative physiology of *Synechococcus* and *Prochlorococcus*: influence of light and temperature on growth, pigments, fluorescence and absorptive properties. Mar Ecol Prog Ser 116:259–275

Moore LR, Rocap G, Chisholm SW (1998) Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. Nature 393:464–467

Moore LR, Post AF, Rocap G, Chisholm SW (2002) Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. Limnol Oceanogr 47:989–996

Moore CM, Mills MM, Langlois R, Milne A, Achterberg EP, LaRoche J, Geider RJ (2008) Relative influence of nitrogen and phosphorus availability on phytoplankton physiology and productivity in the oligotrophic sub-tropical North Atlantic Ocean. Limnol Oceanogr 53:291–305

Morel A, Ahn Y-H, Partensky F, Vaulot D, Claustre H (1993) *Prochlorococcus* and *Synechococcus*: a comparative study of their optical properties in relation to their size and pigmentation. J Mar Res 51:617–649

Morris JJ, Kirkegaard R, Szul MJ, Johnson ZI, Zinser ER (2008) Facilitation of robust growth of *Prochlorococcus* colonies and dilute liquid cultures by "helper" heterotrophic bacteria. Appl Environ Microbiol 74:4530–4534

Mühling M, Fuller NJ, Millard A, Somerfield PJ, Marie D, Wilson WH, Scanlan DJ, Post AF, Joint I, Mann NH (2005) Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton. Environ Microbiol 7:499–508

Mühling M, Fuller NJ, Somerfield PJ, Post AF, Wilson WH, Scanlan DJ, Joint I, Mann NH (2006) High resolution genetic diversity studies of marine *Synechococcus* isolates using *rpoC1*-based restriction fragment length polymorphism. Aquat Microb Ecol 45:263–275

Murphy LS, Haugen EM (1985) The distribution and abundance of phototrophic ultraplankton in the North Atlantic. Limnol Oceanogr 30:47–58

Odate T, Fukuchi M (1995) Physical and chemical properties of surface water in the Southern Ocean in summer 1991/92. Proc NIPR Symp Polar Biol 8:77–85

Olson RJ, Chisholm SW, Zettler ER, Armbrust EV (1988) Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry. Deep Sea Res 35:425–440

Olson RJ, Chisholm SW, Zettler ER, Armbrust EV (1990a) Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. Limnol Oceanogr 35:45–58

Olson RJ, Zettler ER, Altabet MA, Dusenberry JA, Chisholm SW (1990b) Spatial and temporal distributions of prochlorophyte picoplankton in the North Atlantic Ocean. Deep Sea Res Part I 37:1033–1051

Osburne MS, Holmbeck BM, Frias-Lopez J, Steen R, Huang K, Kelly L, Coe A, Waraska K, Gagne A, Chisholm SW (2010) UV hyperresistance in *Prochlorococcus* MED4 results from a single base pair deletion just upstream of an operon encoding nudix hydrolase and photolyase. Environ Microbiol 12:1978–1988

Ostrowski M, Mazard S, Tetu SG, Phillippy K, Johnson A, Palenik B, Paulsen IT, Scanlan DJ (2010) PtrA is required for coordinate regulation of gene expression during phosphate stress in a marine *Synechococcus*. ISME J 4:908–921

Paerl RW, Foster RA, Jenkins BD, Montoya JP, Zehr JP (2008) Phylogenetic diversity of cyanobacterial *narB* genes from various marine habitats. Environ Microbiol 10:3377–3387

Paerl RW, Johnson KS, Welsh RM, Worden AZ, Chavez FP, Zehr JP (2011) Differential distributions of *Synechococcus* subgroups across the California current system. Front Microbiol 2:59

Palenik B (2000) Why do isolates of eubacterial species have different growth rates under the same conditions? In: Bell CR, Brylinsky M, Johnson-Green P (eds) Microbial biosystems: new frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada, pp 611–616

Palenik B (2001) Chromatic adaptation in marine *Synechococcus* strains. Appl Environ Microbiol 67:991–994

Palenik B, Haselkorn R (1992) Multiple evolutionary origins of prochlorophytes, the chlorophyll *b* -containing prokaryotes. Nature 355:265–267

Palenik B, Brahamsha B, Larimer FW, Land M, Hauser L, Chain P, Lamerdin J, Regala W, Allen EE, McCarren J, Paulsen I, Dufresne A, Partensky F, Webb EA, Waterbury J (2003) The genome of a motile marine *Synechococcus*. Nature 424:1037–1042

Palenik B, Ren QH, Dupont CL, Myers GS, Heidelberg JF, Badger JH, Madupu R, Nelson WC, Brinkac LM, Dodson RJ, Durkin AS, Daugherty SC, Sullivan SA, Khouri H, Mohamoud Y, Halpin R, Paulsen IT (2006) Genome sequence of *Synechococcus* CC9311: insights into adaptation to a coastal environment. Proc Natl Acad Sci USA 103:13555–13559

Palenik B, Ren Q, Tai V, Paulsen IT (2009) Coastal *Synechococcus* metagenome reveals major roles for horizontal gene transfer and plasmids in population diversity. Environ Microbiol 11:349–359

Partensky F, Garczarek L (2003) The photosynthetic apparatus of chlorophyll *b*- and *d*-containing Oxychlorobacteria. In: Larkum AWD, Douglas SE, Raven JA (eds) Photosynthesis in algae. Kluwer Academic Publishers, Dordrecht, pp 29–62

Partensky F, Garczarek L (2010) *Prochlorococcus*: advantages and limits of minimalism. Ann Rev Mar Sci 2:305–331

Partensky F, Hoepffner N, Li WKW, Ulloa O, Vaulot D (1993) Photoacclimation of *Prochlorococcus* sp. (Prochlorophyta) strains isolated from the North Atlantic and the Mediterranean Sea. Plant Physiol 101:285–296

Partensky F, Blanchot J, Lantoine F, Neveux J, Marie D (1996) Vertical structure of picophytoplankton at different trophic sites of the tropical northeastern Atlantic Ocean. Deep Sea Res Part I 43:1191–1213

Partensky F, Blanchot J, Vaulot D (1999a) Differential distribution and ecology of *Prochlorococcus* and *Synechococcus* in oceanic waters: a review. Bull Inst Oceanogr 19:457–475

Partensky F, Hess WR, Vaulot D (1999b) *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. Microbiol Mol Biol Rev 63:106–127

Penno S, Lindell D, Post AF (2006) Diversity of *Synechococcus* and *Prochlorococcus* populations determined from DNA sequences of the N-regulatory gene *ntcA*. Environ Microbiol 8:1200–1211

Perkins FO, Haas LW, Phillips DE, Webb KL (1981) Ultrastructure of a marine *Synechococcus* possessing spinae. Can J Microbiol 27:218–329

Pickup ZL, Pickup R, Parry JD (2007) Effects of bacterial prey species and their concentration on growth of the amoebae *Acanthamoeba castellanii* and *Hartmannella vermiformis*. Appl Environ Microbiol 73:2631–2634

Polovina JJ, Howell EA, Abecassis M (2008) Ocean's least productive waters are expanding. Geophys Res Lett 35:L03618

Rabouille S, Edwards CA, Zehr JP (2007) Modelling the vertical distribution of *Prochlorococcus* and *Synechococcus* in the North Pacific Subtropical Ocean. Environ Microbiol 9:2588–2602

Richardson TL, Jackson GA (2007) Small phytoplankton and carbon export from the surface ocean. Science 315:838–840

Rippka R, Coursin T, Hess WR, Lichtlé C, Scanlan DJ, Palinska KA, Iteman I, Partensky F, Houmard J, Herdman M (2000) *Prochlorococcus marinus* Chisholm *et al*. 1992 subsp. *pastoris* subsp. nov. strain PCC 9511, the first axenic chlorophyll *a2/b2*-containing cyanobacterium (Oxyphotobacteria). Int J Syst Evol Microbiol 50:1833–1847

Rivers AR, Wisniewski Jakuba R, Webb EA (2008) Iron stress genes in marine *Synechococcus* and the development of a flow cytometric iron stress assay. Environ Microbiol 11:382–396

Robertson BR, Tezuka N, Watanabe MM (2001) Phylogenetic analyses of *Synechococcus* strains (cyanobacteria) using sequences of 16S rDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content. Int J Syst Evol Microbiol 51:861–871

Rocap G, Distel DL, Waterbury JB, Chisholm SW (2002) Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. Appl Environ Microbiol 68:1180–1191

Rocap G, Larimer FW, Lamerdin J, Malfatti S, Chain P, Ahlgren NA, Arellano A, Coleman M, Hauser L, Hess WR, Johnson ZI, Land M,

Lindell D, Post AF, Regala W, Shah M, Shaw SL, Steglich C, Sullivan MB, Ting CS, Tolonen A, Webb EA, Zinser ER, Chisholm SW (2003) Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. Nature 424:1042–1047

Rodrigue S, Malmstrom RR, Berlin AM, Birren BW, Henn MR, Chisholm SW (2009) Whole genome amplification and de novo assembly of single bacterial cells. PLoS One 9:e6864

Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S, Wu DY, Eisen JA, Hoffman JM, Remington K, Beeson K, Tran B, Smith H, Baden-Tillson H, Stewart C, Thorpe J, Freeman J, Andrews-Pfannkoch C, Venter JE, Li K, Kravitz S, Heidelberg JF, Utterback T, Rogers YH, Falcon LI, Souza V, Bonilla-Rosso G, Eguiarte LE, Karl DM, Sathyendranath S, Platt T, Bermingham E, Gallardo V, Tamayo-Castillo G, Ferrari MR, Strausberg RL, Nealson K, Friedman R, Frazier M, Venter JC (2007) The Sorcerer II Global Ocean Sampling expedition: northwest Atlantic through Eastern Tropical Pacific. PLoS Biol 5:398–431

Rusch DB, Martiny AC, Dupont CL, Halpern AL, Venter JC (2010) Characterisation of *Prochlorococcus* clades from iron-depleted oceanic regions. Proc Natl Acad Sci USA 107:16184–16189

Saito MA, Rocap G, Moffett JW (2005) Production of cobalt binding ligands in a *Synechococcus* feature at the Costa Rica upwelling dome. Limnol Oceanogr 50:279–290

Samuel ADT, Petersen JD, Reese TS (2001) Envelope structure of *Synechococcus* sp. WH8113, a non-flagellated swimming cyanobacterium. BMC Microbiol 1:4

Scanlan DJ (2003) Physiological diversity and niche adaptation in marine *Synechococcus*. Adv Microb Physiol 47:1–64

Scanlan DJ, West NJ (2002) Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. FEMS Microbiol Ecol 40:1–12

Scanlan DJ, Bourne JA, Mann NH (1997) A putative transcriptional activator of the Crp/Fnr family from the marine cyanobacterium *Synechococcus* sp. WH7803. J Appl Phycol 8:565–567

Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, Post AF, Hagemann M, Paulsen I, Partensky F (2009) Ecological genomics of marine picocyanobacteria. Microbiol Mol Biol Rev 73:249–299

Shalapyonok A, Olson RJ, Shalapyonok LS (1998) Ultradian growth in *Prochlorococcus* spp. Appl Environ Microbiol 64:1066–1069

Shapiro LP, Haugen EM (1988) Seasonal distribution and temperature tolerance of *Synechococcus* in Boothbay Harbor, Maine. Estuar Coast Shelf Sci 26:517–525

Shi Y, Tyson GW, Delong EF (2009) Metatranscriptomics reveals unique microbial small RNAs in the ocean's water column. Nature 459:266–272

Shimada A, Nishijima M, Maruyama T (1995) Seasonal abundance of *Prochlorococcus* in Suruga Bay, Japan in 1992–1993. J Oceanogr 51:289–300

Sieracki ME, Haugen EM, Cucci TL (1995) Overestimation of heterotrophic bacteria in the Sargasso Sea: direct evidence by flow and imaging cytometry. Deep Sea Res Part I 42:1399–1409

Six C, Thomas JC, Brahamsha B, Lemoine Y, Partensky F (2004) Photophysiology of the marine cyanobacterium *Synechococcus* sp. WH8102, a new model organism. Aquat Microb Ecol 35:17–29

Six C, Thomas JC, Garczarek L, Ostrowski M, Dufresne A, Blot N, Scanlan DJ, Partensky F (2007) Diversity and evolution of phycobilisomes in marine *Synechococcus* spp.: a comparative genomics study. Genome Biol 8:R259

Ŝmarda J, Ŝmajs D, Komrska J, Krzyžánek V (2002) S-layers on cell walls of cyanobacteria. Micron 33:257–277

Snyder DS, Brahamsha B, Azadi P, Palenik B (2009) Structure of compositionally simple lipopolysaccharide from marine *Synechococcus*. J Bacteriol 191:5499–5509

Sode K, Oonari R, Oozeki M (1997) Induction of a temperate marine cyanophage by heavy metal. J Mar Biotechnol 5:178–180

Steglich C, Behrenfeld M, Koblizek M, Claustre H, Penno S, Prasil O, Partensky F, Hess WR (2001) Nitrogen deprivation strongly affects photosystem II but not phycoerythrin level in the divinyl-chlorophyll *b*-containing cyanobacterium *Prochlorococcus marinus*. Biochim Biophys Acta 1503:341–349

Steglich C, Mullineaux CW, Teuchner K, Hess WR, Lokstein H (2003a) Photophysical properties of *Prochlorococcus marinus* SS120 divinyl chlorophylls and phycoerythrin *in vitro* and *in vivo*. FEBS Lett 553:79–84

Steglich C, Post AF, Hess WR (2003b) Analysis of natural populations of *Prochlorococcus* spp. in the northern Red Sea using phycoerythrin gene sequences. Environ Microbiol 5:681–690

Steglich C, Frankenberg-Dinkel N, Penno S, Hess WR (2005) A green light-absorbing phycoerythrin is present in the high-light-adapted marine cyanobacterium *Prochlorococcus* sp. MED4. Environ Microbiol 7:1611–1618

Steglich C, Futschik M, Rector T, Steen R, Chisholm SW (2006) Genome-wide analysis of light sensing in *Prochlorococcus*. J Bacteriol 188:7796–7806

Steglich C, Futschik ME, Lindell D, Voss B, Chisholm SW, Hess WR (2008) The challenge of regulation in a minimal phototroph: non-coding RNAs in *Prochlorococcus*. PLoS Genet 4:e1000173

Steinberg DK, Carlson CA, Bates NR, Johnson RJ, Michaels AF, Knap AH (2001) Overview of the US JGOFS Bermuda Atlantic Time-series Study (BATS): a decade-scale look at ocean biology and biogeochemistry. Deep Sea Res Part II 48:1405–1447

Stockner JW (1988) Phototrophic picoplankton: an overview from marine, freshwater ecosystems. Limnol Oceanogr 33:765–775

Strom SL, Brahamsha B, Fredrikson KA, Apple JK, Rodríguez AG. (2012) A giant cell surface protein in *Synechococcus* WH8102 inhibits feeding by a dinoflagellate predator. Environ Microbiol 14:807–816

Stomp M, Huisman J, de Jongh F, Veraart AJ, Gerla D, Rijkeboer M, Ibelings BW, Wollenzien UIA, Stal LJ (2004) Adaptive divergence in pigment composition promotes phytoplankton biodiversity. Nature 432:104–107

Stomp M, Huisman J, Vörös L, Pick FR, Laamanen M, Haverkamp T, Stal LJ (2007) Colourful coexistence of red and green picocyanobacteria in lakes and seas. Ecol Lett 10:290–298

Stuart RK, Dupont CL, Johnson DA, Paulsen IT, Palenik B (2009) Coastal strains of marine *Synechococcus* species exhibit increased tolerance to copper shock and a distinctive transcriptional response relative to those of open-ocean strains. Appl Environ Microbiol 75:5047–5057

Su ZC, Dam P, Chen X, Olman V, Jiang T, Palenik B, Xu Y (2003) Computational interference of regulatory pathways in microbes: an application to phosphorus assimilation pathways in *Synechococcus* sp. WH8102. Genome Inform 14:3–13

Su Z, Olman V, Mao F, Xu Y (2005) Comparative genomics analysis of NtcA regulons in cyanobacteria: regulation of nitrogen assimilation and its coupling to photosynthesis. Nucleic Acids Res 33:5156–5171

Su ZC, Mao FL, Dam P, Wu HW, Olman V, Paulsen IT, Palenik B, Xu Y (2006) Computational inference and experimental validation of the nitrogen assimilation regulatory network in the cyanobacterium *Synechococcus* sp. WH 8102. Nucleic Acids Res 34:1050–1065

Su ZC, Olman V, Xu Y (2007) Computational prediction of Pho regulons in cyanobacteria. BMC Genomics 8:156

Tai V, Palenik B (2009) Temporal variation of *Synechococcus* clades at a coastal Pacific Ocean monitoring site. ISME J 3:903–915

Tai V, Paulsen IT, Phillippy K, Johnson DA, Palenik B (2009) Whole-genome microarray analyses of *Synechococcus–Vibrio* interactions. Environ Microbiol 11:2698–2709

Tang EPY, Vincent WF (1999) Strategies of thermal adaptation by high-latitude cyanobacteria. New Phytol 142:315–323

Tetu SG, Brahamsha B, Johnson DA, Tai V, Phillippy K, Palenik B, Paulsen IT (2009) Microarray analysis of phosphate regulation in

the marine cyanobacterium *Synechococcus* sp. WH8102. ISME J 3:835–849

Thomas EV, Phillippy KH, Brahamsha B, Haaland DM, Timlin JA, Elbourne LDH, Palenik, Paulsen IT (2009) Statistical analysis of microarray data with replicated spots: a case study with *Synechococcus* WH8102. Comp Funct Genomics, Article ID 950171

Ting CS, Rocap G, King J, Chisholm SW (2002) Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. Trends Microbiol 10:134–142

Ting CS, Hsieh C, Sundararaman S, Mannella C, Marko M (2007) Cryo-electron tomography reveals the comparative three-dimensional architecture of *Prochlorococcus*, a globally important marine cyanobacterium. J Bacteriol 189:4485–4493

Ting CS, Ramsey ME, Wang YL, Frost AM, Jun E, Durham T (2009) Minimal genomes, maximal productivity: comparative genomics of the photosystem and light-harvesting complexes in the marine cyanobacterium *Prochlorococcus*. Photosynth Res 101:1–19

Toledo G, Palenik B (1997) *Synechococcus* diversity in the California Current as seen by RNA polymerase (*rpoC1*) gene sequences of isolated strains. Appl Environ Microbiol 63:4298–4303

Toledo G, Palenik B (2003) A *Synechococcus* serotype is found preferentially in surface marine waters. Limnol Oceanogr 48:1744–1755

Toledo G, Palenik B, Brahamsha B (1999) Swimming marine *Synechococcus* strains with widely different photosynthetic pigment ratios form a monophyletic group. Appl Environ Microbiol 65:5247–5251

Tolonen AC, Aach J, Lindell D, Johnson ZI, Rector T, Steen R, Church GM, Chisholm SW (2006a) Global gene expression of *Prochlorococcus* ecotypes in response to changes in nitrogen availability. Mol Syst Biol 2:53

Tolonen AC, Liszt GB, Hess WR (2006b) Genetic manipulation of *Prochlorococcus* strain MIT9313: green fluorescent protein expression from an RSF1010 plasmid and Tn5 transposition. Appl Environ Microbiol 72:7607–7613

Urbach E, Robertson DL, Chisholm SW (1992) Multiple evolutionary origins of prochlorophytes within the cyanobacterial radiation. Nature 355:267–270

Urbach E, Scanlan DJ, Distel DL, Waterbury JB, Chisholm SW (1998) Rapid diversification of marine picophytoplankton with dissimilar light-harvesting structures inferred from sequences of *Prochlorococcus* and *Synechococcus* (Cyanobacteria). J Mol Evol 46:188–201

Uysal Z (2000) Pigments, size and distribution of *Synechococcus* spp. in the Black Sea. J Mar Syst 24:313–326

Vaulot D, Patensky F, Neveux J, Mantoura RFC, Llewellyn C (1990) Winter presence of prochlorophytes in surface waters of the northwestern Mediterranean Sea. Limnol Oceanogr 35:1156–1164

Vaulot D, Marie D, Olson RJ, Chisholm SW (1995) Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the equatorial Pacific Ocean. Science 268:1480–1482

Vaulot D, LeGall F, Marie D, Guillou L, Partensky F (2004) The Roscoff Culture Collection (RCC): a collection dedicated to marine picoplankton. Nova Hedwig 79:49–70

Veldhuis MJW, Kraay GW (1993) Cell abundance and fluorescence of picoplankton in relation to growth irradiance and nitrogen availability in the Red Sea. Neth J Sea Res 31:135–145

Venter JC, Remington K, Heidelberg JF, Halpern L, Rusch D, Eisen JA, Wu DY, Paulsen I, Nelson KE, Nelson W, Fouts DE, Levy S, Knap AH, Lomas MW, Nealson K, White O, Peterson J, Hoffman J, Parsons R, Baden-Tillson H, Pfannkoch C, Rogers YH, Smith HO (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66–74

Vincent WF, Bowman J, Powell L, McMeekin T (2000) Phylogenetic diversity of picocyanobacteria in Arctic and Antarctic ecosystems. In: Brylinsky M, Bell C, Johnson-Green P (eds) Microbial biosystems: new frontiers. Proceedings of the 8th international symposium on microbial ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada, pp 317–322

Waleron M, Waleron K, Vincent WF, Wilmotte A (2007) Allochthonous inputs of riverine picocyanobacteria to coastal waters in the Arctic Ocean. FEMS Microbiol Ecol 59:356–365

Waterbury JB, Rippka R (1989) Subsection 1. Order Chroococcales Wettstein 1924, emend. Rippka et al. *1*979. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams and Wilkins, Baltimore, pp 1728–1746

Waterbury JB, Watson SW, Guillard RRL, Brand LE (1979) Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. Nature 277:293–294

Waterbury JB, Willey JM, Franks DG, Valois FW, Watson SW (1985) A cyanobacterium capable of swimming motility. Science 230:74–76

Waterbury JB, Watson SW, Valois FW, Franks DG (1986) Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. Can Bull Fish Aquat Sci 472:71–120

Wawrik B, Paul JH, Campbell L, Griffin D, Houchin L, Fuentes-Ortega A, Muller-Karger F (2003) Vertical structure of the phytoplankton community associated with a coastal plume in the Gulf of Mexico. Mar Ecol Prog Ser 251:87–101

Wells ML, Price NM, Bruland KW (1994) Iron limitation and the cyanobacterium *Synechococcus* in equatorial Pacific waters. Limnol Oceanogr 39:1481–1486

West NJ, Scanlan DJ (1999) Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern North Atlantic Ocean. Appl Environ Microbiol 65:2585–2591

West NJ, Lebaron P, Strutton PG, Suzuki MT (2011) A novel clade of *Prochlorococcus* found in high nutrient low chlorophyll waters in the South and Equatorial Pacific Ocean. ISME J 5:933–944

West NJ, Schönhuber WA, Fuller NJ, Amann RI, Rippka R, Post AF, Scanlan DJ (2001) Closely related *Prochlorococcus* genotypes show remarkably different depth distributions in two oceanic regions as revealed by *in situ* hybridization using 16S rRNA-targeted oligonucleotides. Microbiology 147:1731–1744

Wilmotte A, Demonceau C, Goffart A, Hecq J-H, Demoulin V, Crossley AC (2002) Molecular and pigment studies of the picophytoplankton in a region of the Southern Ocean (42–54°S, 141–144°E) in March 1998. Deep Sea Res Part II 49:3351–3363

Wilson A, Ajlani G, Verbavatz JM, Vass I, Kerfeld CA, Kirilovsky D (2006) A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria. Plant Cell 18:992–1007

Wilson A, Boulay C, Wilde A, Kerfeld CA, Kirilovsky D (2007) Light-induced energy dissipation in iron-starved cyanobacteria: roles of OCP and IsiA proteins. Plant Cell 19:656–672

Wood AM, Horan PK, Muirhead K, Phinney DA, Yentsch CM, Waterbury JB (1985) Discrimination between types of pigments in marine *Synechococcus* spp. by scanning spectroscopy, epifluorescence microscopy, and flow cytometry. Limnol Oceanogr 30:1303–1315

Wood AM, Phinney DA, Yentsch CS (1998) Water column transparency and the distribution of spectrally distinct forms of phycoerythrin containing organisms. Mar Ecol Prog Ser 162:25–31

Wood AM, Lipsen M, Coble P (1999) Fluorescence-based characterisation of phycoerythrin-containing cyanobacterial communities in the Arabian Sea during the northeast and early southwest monsoon (1994–1995). Deep Sea Res Part II 46:1769–1790

Worden AZ, Binder BJ (2003) Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synechococcus* populations in oligotrophic environments. Aquat Microb Ecol 30:159–174

Wyman M, Bird C (2007) Lack of control of nitrite assimilation by ammonium in an oceanic picocyanobacterium, *Synechococcus* sp. strain WH8103. Appl Environ Microbiol 73:3028–3033

Zinser ER, Coe A, Johnson ZI, Martiny AC, Fuller NJ, Scanlan DJ, Chisholm SW (2006) *Prochlorococcus* ecotype abundances in the

North Atlantic Ocean as revealed by an improved quantitative PCR method. Appl Environ Microbiol 72:723–732

Zinser ER, Johnson ZI, Coe A, Karaca E, Veneziano D, Chisholm SW (2007) Influence of light and temperature on *Prochlorococcus* ecotype distributions in the Atlantic Ocean. Limnol Oceanogr 52:2205–2220

Zinser ER, Lindell D, Johnson ZI, Futschik ME, Steglich C, Coleman ML, Wright MA, Rector T, Steen R, McNulty N, Thompson LR, Chisholm SW (2009) Choreography of the transcriptome, photophysiology, and cell cycle of a minimal photoautotroph, *Prochlorococcus*. PLoS One 4:e5135

Zohary T, Robarts RD (1998) Experimental study of microbial P limitation in the eastern Mediterranean. Limnol Oceanogr 43:387–395

Zubkov MV, Tarran GA (2005) Amino acid uptake of *Prochlorococcus* spp. in surface waters across the South Atlantic Subtropical Front. Aquat Microb Ecol 40:241–249

Zubkov MV, Sleigh MA, Burkill PH, Leakey RJG (2000) Picoplankton community structure on the Atlantic Meridional Transect: a comparison between seasons. Prog Oceanogr 45:369–386

Zwirglmaier K, Heywood JL, Chamberlain K, Woodward EMS, Zubkov MV, Scanlan DJ (2007) Basin-scale distribution patterns of picocyanobacterial lineages in the Atlantic Ocean. Environ Microbiol 9:1278–1290

Zwirglmaier K, Jardillier L, Ostrowski M, Mazard S, Garczarek L, Vaulot D, Not F, Massana R, Ulloa O, Scanlan DJ (2008) Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ Microbiol 10:147–161

Zwirglmaier K, Spence E, Zubkov MV, Scanlan DJ, Mann NH (2009) Differential grazing of two heterotrophic nanoflagellates on marine *Synechococcus* strains. Environ Microbiol 11:1767–1776

Cyanophages

21

Nicholas H. Mann and Martha R.J. Clokie

Contents

N.H. Mann (✉)
School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK
e-mail: N.H.Mann@warwick.ac.uk

M.R.J. Clokie
Department of Infection, Immunity and Inflammation, University of Leicester, Maurice Shock Medical Sciences Building, PO Box 138, Leicester, LE1 9HN, UK
e-mail: mrjc1@le.ac.uk

Summary

Cyanophages, phages that infect cyanobacteria, are amongst the most abundant biological entities on the planet. They exert profound influences on the community structures of their hosts and on the biogeochemical cycles in which their hosts participate. Indeed, some cyanophages may directly contribute to the carbon cycle by a "viral" form of photosynthesis. The impact of cyanophages on natural populations has several dimensions. Cyanophages may cause population collapses, but where standing stocks are maintained, cyanophages may influence genetic diversity and strain succession. The relationship is reciprocal and changes in host diversity will feedback into fluctuations in phage diversity. It is not clear yet to what extent phage infection competes with protistan grazing as the two major biotic causes of cyanobacterial mortality, but it is becoming apparent that there are subtle interactions between these two processes. This is in part related to the potential ability of cyanophages to alter the phenotype of the infected cell, not only via lysogeny, but also during the normal lytic infection cycle and during pseudolysogeny. Genomic analysis of cyanophages is revealing the extent to which cyanophages can modify the metabolism of the host cell via the acquisition of host-derived genes such as those involved in photosynthesis, carbon metabolism and nutrient acquisition.

21.1 Introduction

It is almost a century since the discovery of bacteriophages, viruses that infect bacteria, and almost 70 years since their particulate nature was confirmed, yet our understanding of their ecology is still largely in its infancy. There is a rapidly growing appreciation that bacteriophages exert major influences on biogeochemical cycles and the dynamics and composition of bacterial populations as well as acting as vectors for horizontal gene transfer in the natural environment. Bacteriophages are now recognized to be the most abundant

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biological entities in the biosphere with an estimated total population of between 10^{30} and 10^{32} (Brussow and Kutter 2005) and genomic and metagenomic analyses have revealed that they constitute the largest reservoir of uncharacterized genetic diversity (Pedulla et al. 2003).

The first steps in this recognition of the ecological significance of phages were made by studies on aquatic environments that provided the first quantitative estimates of viral abundance (Bergh et al. 1989) and over recent years phage diversity, mainly in the marine environment, has been a major area of study, coupled with attempts to assess the gross contribution of phages to bacterial mortality. As a result of this interest it has become clear that cyanophages are a major component of the marine virosphere (Rohwer et al. 2009; Ghai et al. 2010). Studies on cyanophages, however, were initially focussed on fresh water environments and isolation of the first cyanophage, infecting strains of *Lyngbya, Plectonema* and *Phormidium*, was reported in 1963 (Safferman and Morris 1963). The early work on cyanophages has been reviewed extensively (Padan and Shilo 1973; Safferman 1973; Martin and Benson 1988; Suttle 2000), so consequently this chapter focuses on aspects of cyanophage biology that affect the composition and dynamics of cyanobacterial populations or the ecophysiology of individual cyanobacterial cells. Other aspects of cyanophage biology are dealt with in recent reviews by Mann (2003, 2006).

Phages in general and cyanophages in particular, like all viruses, are obligate intracellular parasites and they are extremely heterogeneous in their physico-chemical and biological properties. Phage virion morphologies include tailed, polyhedral, filamentous and pleomorphic, though the vast majority (96%) are tailed and while most phages contain dsDNA, ssDNA, ssRNA or dsRNA are found in some phages (Ackermann 2005; Ackermann 2007). Almost all of the cyanophages that have been isolated and characterized to date are members of the Caudovirales i.e. tailed phages with dsDNA genomes (Ackermann and DuBow 1987). Whilst 13 families are currently recognized within the Caudovirales, cyanophages are only represented in three of these families; the Myoviridae have a long contractile tail, the Siphoviridae have a long non-contractile tail and the Podoviridae have a short non-contractile tail (Fig. 21.1a–c). Although the terms have no taxonomic authority, cyanophages are commonly described as cyanomyoviruses, cyanosiphoviruses and cyanopodoviruses. Other morphological forms of cyanophages almost certainly exist, though few have been isolated. Examples of a novel cyanophage morphology are the three filamentous phages recently isolated that infect *Microcystis, Anabaena* and some potential *Planktothrix* strains (Deng and Hayes 2008). There is a report of an inducible prophage with an ssDNA genome (McDaniel et al. 2006).

For the last decade it has been thought by some members of the bacteriophage community that there are inherent problems with a phenetic approach to phage classification (Lawrence et al. 2002) and that a taxonomy based on genomic analyses might be more appropriate (Rohwer and Edwards 2002; Lavigne et al. 2009). Aspects of the molecular taxonomy of cyanophages are discussed in 21.5. Most scientists follow the convention suggested by Suttle whereby the first letter represents the genus of the cyanobacterium they infect, the second two letters represent a geographical location code signifying where the phage was isolated, the final letter indicates the phage morphology and the number shows what number phage from that particular family it was, isolated from that site (Suttle 2000). To give an example, the phage S-SKM4 in Fig 21.1 A is so named because S indicates it infects *Synechococcus*, SK it was isolated from St. Kilda, M is a myovirus and 4 was the fourth *Synechococcus* myovirus isolated from St. Kilda.

Despite morphological differences there are common features to the initial interactions of phages with potential host cells; these include adsorption, introduction of the viral genome into the cell and subsequent expression of viral genes. From this point, however, specific phages exhibit different life cycles. Oligately lytic phages, sometimes referred as virulent phages, will kill their bacterial host, following infection, by lysing the infected cell to release progeny phages. The period following entry of the genome into the infected cell before mature progeny phages are assembled is termed the eclipse period and the time from genome entry to the subsequent progeny release into the environment via lysis of the host cell defines the latent period. The number of progeny phage produced per infected cell is termed the burst size. Temperate phages may also kill the host cell following infection, but in addition are also capable of forming a long term stable relationship with the host known as lysogeny. This relationship commonly involves the phage becoming integrated into the genome of the host as a prophage. A chronic infection is one where the host cell is infected and progeny phage are released without lysis of the host. There are no known cases of chronic infection by cyanophages. There are other rather more controversial life style variations such as "pseudolysogeny" and the "carrier state", which in fact may be of considerable significance in the natural environment. These additional aspects of phage life style have been extensively discussed by Abedon (Abedon 2009). The definition of pseudolysogeny accepted here is that it represents a quiescent state following phage genome introduction into the host during which the typical infection process is not initiated and this idea is further explored in Sect. 21.5.2. A general overview of basic phage biology is provided by Guttman et al. (2005).

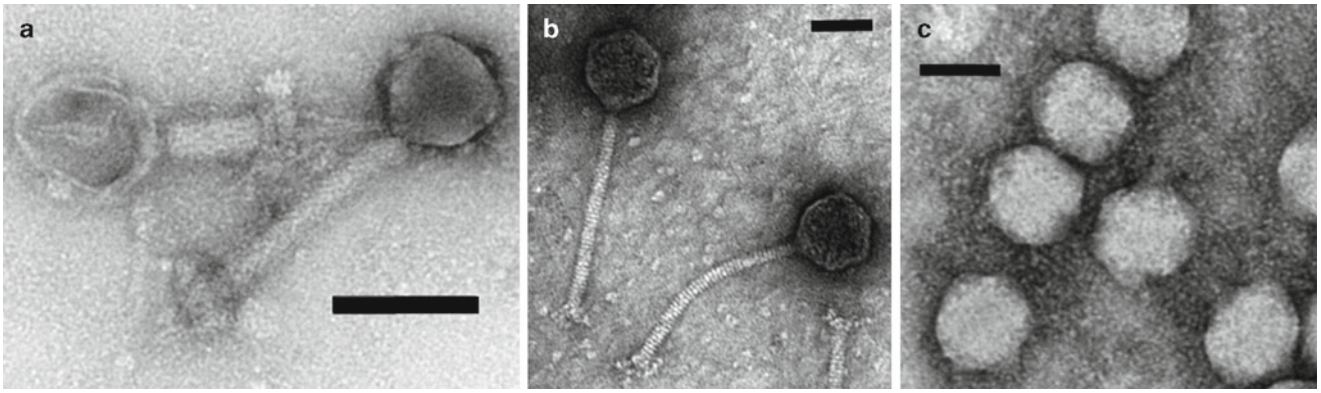

Fig. 21.1 (**a**) *Synechococcus* cyanomyoviruses (S-SKM4), the phage on the *left* has a contracted tail and an empty head of DNA, and the right-hand phage has an extended tail and a full head of DNA, note the base plate with extending tail fibres at the bottom of the particles; scale bar = 100 nm. (Image taken by Joe Morley and Stefan Hyman at the School of Biological Sciences, University of Leicester). (**b**) *Acaryochloris* cyanosiphoviruses (A-HIS2) with long non-contractile tails. (By courtesy of Yi Chan and Martha Clokie) scale bar = 50 nm. (**c**) *Synechococcus* cyanopodoviruses (S-CBP1) with very small tails. scale bar = 50 nm. (By courtesy of Feng Chen, University of Maryland, USA)

21.2 Impact of Cyanophages on Natural Populations

Following the isolation of the first cyanophages in the 1960s there was speculation that they could be used to control blooms of nuisance cyanobacteria and there was considerable interest is characterizing the impact of cyanophages on natural populations of their hosts. However, no significant success was achieved in control of cyanobacterial blooms and this, in part, led to a decline in such cyanophage ecology studies. Interest was reawakened by the discovery in 1989 that viruses are numerous in aquatic environments (Bergh et al. 1989) and that strains of unicellular bacteria that they potentially infect are abundant in the world's oceans and therefore contribute significantly to global carbon cycling (Johnson and Sieburth 1979; Waterbury et al. 1979; Chisholm et al. 1988). It is now becoming clear that cyanophages affect the dynamics and clonal composition of natural cyanobacterial assemblages as well as influencing biogeochemical cycles. In addition to cyanophages being abundant in the bulk aqueous phase of marine and freshwater systems they have also been found as a component of the viral communities associated with healthy and bleaching corals (Marhaver et al. 2008), modern stromatolites and thrombolites (Desnues et al. 2008) and Arctic microbial mats (Chap. 13). It is a natural facet of phage:host interactions that the impact of phages on the dynamics and composition of a host population that may be genetically diverse will feed back to the dynamics and composition of the phage population. A lytic phage will infect and kill the most abundant sensitive host in a population allowing less abundant, but resistant, strains of the host to dominate the population, when they too will become the target for other phages – this is formulated as the "kill the winner hypothesis" (Thingstad 2000; Winter et al. 2010). In addition to being able to enumerate phage and host abundances, molecular approaches have now provided the tools to analyse diversity within the phage and host populations. Indeed, metagenomic approaches looking at differences between strains of the same bacterial species are consistent with the idea that phage predation actually generates and maintains diversity in microbial populations (Rodriguez-Valera et al. 2009).

21.3 Theoretical Considerations of the Impact of Cyanophages on Cyanobacteria

There are several aspects of the interaction between cyanophages and their hosts that need careful consideration from a theoretical point of view in order to be able to assess the experimental and observational data that suggesting that phages impact natural population dynamics In particular it is important to consider the frequency with which phages may encounter and adsorb to a host cell, together with their ability to cause a productive infection, which may be influenced by both genetic and physiological features of the host. The rate at which the host population declines and the phage population increases and the levels at which they are maintained are primarily determined by the adsorption rate, the burst size, the length of the latent period, the rate of host growth, the host population density and the phage population density (Levin and Bull 2004).

A key question relating to the impact of an obligately lytic phage on a host population is whether or not there is a critical "replication threshold" (Wiggins and Alexander 1985) or

"proliferation threshold" (Payne and Jansen 2000) below which the phage will not exert a selection pressure on the host. Experimental evidence using heterotrophic hosts suggests that this threshold is around 10^4 cells mL^{-1} (Wiggins and Alexander 1985). However, more recently, the threshold concept was called into question using mathematical modelling with experimental support (Kasman et al. 2002). One of the key reasons for this discrepancy has been suggested to be that the rate of phage loss *in vitro* is much lower than that in the natural environment (Payne and Jansen 2002), but also because the threshold is only observable when the phage concentration is below an "inundation threshold". Using the phage-host contact rate predictions of transport theory (Murray and Jackson 1992) combined with the measurement of decay rates for natural populations of *Synechococcus* phages (Suttle and Chan 1994; Garza and Suttle 1998) it becomes possible to calculate a theoretical threshold at which the population of a lytic phage would increase rather than just be maintained (Mann 2003).

Calculation of the threshold is very sensitive to values used for decay rate and burst size, but yields threshold values in the range 90–6,000 cells mL^{-1}. There is some observational support for these values. In the case of marine *Synechococcus*, Suttle and Chan, studying natural population of *Synechococcus*; found that there was a marked increase in phage numbers above a host threshold of ca. 1×10^3 cells mL^{-1} (Suttle and Chan 1994), though subsequent data indicated a threshold of 10^4 cells mL^{-1} (Rodda 1996) as cited by (Suttle 2000) which agrees well with data of Wiggins and Alexander (Wiggins and Alexander 1985). The topics of contact rates, decay and thresholds are more extensively discussed by Mann (Mann 2003). These discussions apply to single celled hosts growing planktonically and would not apply to filamentous hosts, however, a mathematical model of viral impact on light-limited *Limnothrix* sp. populations in shallow lakes predicted that burst sizes greater than 200–400 would result in host extinction, whereas smaller burst sizes would allow stable coexistence of host and phage populations (Gons et al. 2006). The ecological implication of this analysis might be that the transition from phosphorus-limited to light-limited cyanobacterial growth could trigger the *Limnothrix* population collapse.

21.4 Host Ranges

A very significant component of any consideration of the impact of phages on cyanobacterial assemblages is host range and the related phenomena of sensitivity and resistance (see Sect. 21.4). The clonal diversity of cyanobacterial assemblages coupled with population density represent important factors in phage ecology. The observation that, in general, phage host range appears to be negatively correlated with host abundance has been investigated using optimal foraging theory (Guyader and Burch 2008). Comparison of generalist and specialist phages confirmed that specialist phages are favoured at high host densities, but that genetic factors rather than foraging strategy constrain the evolution of specialist phages. Consequently, host range studies must take into account host population densities in any attempt to establish the ecological role of phages in determining host dynamics and clonal composition. One caveat applying to all studies of phage host ranges is that they may be very sensitive to which host strain a phage was propagated on prior to assessment of host range since the hosts may possess different restriction-modification systems.

There have been many problems associated with establishing the host range of cyanophages infecting both filamentous and unicellular freshwater cyanobacteria arising largely, though not exclusively, from problems with cyanobacterial taxonomy and these are excellently discussed by Suttle (Suttle 2000). The LPP group of phages (so termed because of their infection of strains of *Lyngbya, Plectonema* and *Phormidium*) are serologically unrelated podoviruses, but were thought to have the same host range, indeed, it was thought at one time that the hosts might be classified in the same order and family (Padan and Shilo 1973). Furthermore, there are complications implicit in the early host range studies and an attempt was made to resolve these problems through the most extensive LPP phage host range analysis (Johnson and Potts 1985). The sensitivity of 33 cyanobacterial strains and variants to infection by the LPP-1 archetype, 5 LPP-1 serotypes, 6 LPP-2 serotypes and 8 novel LPP isolates was assessed. The results confirmed that the phage host range is restricted to certain filamentous, unbranched non-heterocystous strains, however, the LPP designation was felt to reflect inadequacies in cyanobacterial taxonomy rather than a true polygeneric host range. The data indicated the presence of distinct sub-grouping with distinct patterns of phage sensitivity within the LPP group. A similar story surrounds phages assigned to the A, AN, N and NP groups based on their ability to infect strains assigned to the genera *Anabaena, Nostoc* and *Plectonema* (Suttle 2000) i.e. their polygeneric host range was apparently a reflection of a dysfunctional taxonomy. Similar problems exist with phages infecting unicellular freshwater cyanobacteria. However, there is one study in which the host ranges of 35 cyanophages isolated on strains belonging to *Microcystis, Anabaena* and *Planktothrix* unambiguously indicated a polygeneric host range (Deng and Hayes 2008).

There is less of a problem with the analysis of phages infecting the abundant unicellular marine *Synechococcus* and *Prochlorococcus*. The current phylogenetic status of marine strains of these two genera is extensively discussed in Chap. 20 and, except where otherwise stated, the term marine *Synechococcus* is used to refer to *Synechococcus*

sub-cluster 5.1, "which is the dominant *Synechococcus* group within the euphotic zone of open ocean waters, comprising several taxonomically distinct clades" and containing phycoerythrin as the major light-harvesting pigment. Sub-cluster 5.2 strains possess phycocyanin, but lack phycoerythrin. The first studies on the host range of phages infecting marine *Synechococcus* sub-cluster 5.1 strains found considerable variations (Waterbury and Valois 1993). Some phages infected as many as 10 of the 13 strains tested, whereas others would infect only the strain used for isolation. One phage isolated on a *Synechococcus* sub-cluster 5.1 strain, also infected a sub-cluster 5.2 strain (WH8101). None of the phages infected the freshwater *Synechococcus* sp. PCC 6307. Another study involving the isolation of phages on both sub-cluster 5.1 and 5.2 hosts found that host range was not correlated with the geographical locations where the phages and hosts were isolated (Suttle and Chan 1993). Phages from all three tailed cyanophage families were isolated that infected *Synechococcus* strains, but all of the phages capable of infecting sub-cluster 5.1 strains were myoviruses. One of their isolates, a myovirus (S-PWM3), infected a green *Synechococcus* (presumably sub-cluster 5.2) as well as three sub-cluster 5.1 strains. Again no infectivity against freshwater strains was observed. Thus, from these studies myoviruses appeared to exhibit broader host ranges.

Analysis of phages from estuarine sources also isolated on sub-cluster 5.1 and 5.2 hosts again revealed that phages infecting sub-cluster 5.1 strains had a broader host range and that there were phages infecting hosts from both sub-clusters. However, when phages were isolated on estuarine *Synechococcus* hosts the podoviruses and siphoviruses were found to be highly host-specific (Wang and Chen 2008). These observations have been extended by the discovery that there are marine phages that can infect both *Prochlorococcus* and *Synechococcus* (Sullivan et al. 2003). Furthermore, there was a clear distinction between phages which infected the strains of the high-light clade of *Prochlorococcus*, which were highly strain-specific podoviruses and those which infected strains of *Synechococcus* sub-cluster 5.1 and the low-light clade of *Prochlorococcus*, which were broader host range myoviruses. The clear distinction between marine and freshwater cyanophages was muddied by the discovery of the pervasive distribution of phages in Lake Erie that infect the marine sub-cluster 5.1 strain WH7803 (Wilhelm et al. 2006). Similar observations have been made with phages that are capable of infecting the heterocystous filamentous cyanobacterium *Nodularia spumigena* isolated from the brackish Baltic Sea (Jenkins and Hayes 2006). The phages, which included both myoviruses and siphoviruses, exhibited variable host ranges with some being able to infect five genotypically distinct strains of *Nodularia spumigena* and others infecting only one strain.

21.4.1 Evidence of Impact

Whilst techniques such as flow cytometry, epifluorescence microscopy and electron microscopy enable us to enumerate total virus populations in a given environment, the enumeration of specific phages still relies primarily on the conventional microbiological approaches of plaque assay on a solid medium, or use of the most probable number method in liquid culture. Both these methods require the use of a host strain sensitive to phage infection and which is culturable in the laboratory. Furthermore, natural assemblages of hosts and phages are genetically diverse and it is unlikely that one strain of a particular host will be sensitive to infection by all the phages capable of infecting that particular host. Thus these direct approaches to estimating host-specific phage abundance will produce an underestimate and even if multiple host strains were used it would not be easy to establish whether phage titres were additive or overlapping. The nature and extent of this problem becomes apparent from a study using a fluorescently stained phages capable of infecting a relatively broad host range of laboratory-cultured marine *Synechococcus* strains (Hennes et al. 1995). The phages were observed to attach to only about 3% of the *Synechococcus* cells in two samples from the Gulf of Mexico. A further complication arises from the observation that phages would only attach to about 10% of the cells of a single host strain of *Synechococcus* in laboratory culture suggesting that only a proportion of the cells of genetically homogeneous population might be expressing the phage receptor at any time. Similar phenomena were also observed by Jia et al. (2010) who showed that a very low proportion of some cyanophages actually bound to their hosts. A final level of complexity arises from the fact that there may be a diel component to phage infection of cyanobacteria (Clokie et al. 2006c). These caveats must be borne in mind in considering analyses of host-specific phage abundances.

21.4.1.1 Freshwater Cyanophage Studies

Following the first isolation of cyanophages there were many attempts to establish their ecological role in determining the dynamics of cyanobacterial populations (Shane 1971; Shane et al. 1972; Cannon et al. 1974; Fallon and Brock 1979; Coulombe and Robinson 1981; Martin and Benson 1988). Although there had been interest in the potential of phages to be used to control noxious cyanobacterial blooms there are very few accounts of their successful use. There are two reports by Martin and co-workers (Martin et al. 1978; Martin 1982) on the use of phages to control cyanobacteria in experimental field enclosures and one on the use of the LPP phage to control *Plectonema* populations in experimental ponds (Desjardins and Olsen 1983) as cited by (Philips et al. 1990). LPP phages capable of infecting strains of the closely related genera *Lyngbya*, *Plectonema* and *Phormidium* have been

isolated from a number of fresh water sources including waste stabilization ponds (Safferman and Morris 1963), fish ponds (Padan and Shilo 1969) and rice fields (Singh 1973). Counts as high as 270 pfu mL^{-1} were reported for waste stabilization ponds (Safferman and Morris 1967).

Measurement of LPP phages numbers in Israeli fish ponds over a year typically gave titres ranging from single figures to several tens of pfu mL^{-1}, however, increases in titre to several thousands of pfu mL^{-1} were occasionally observed when intensive cyanobacterial blooms occurred (Padan and Shilo 1969). Much more recently there has been interest in the role that phages might play in the ecological dynamics of *Microcystis aeruginosa*, given the potential of the organism to form blooms coupled with the productions of potent toxins, the microcystins. Seasonal changes in the populations of cyanophages infectious to *M. aeruginosa* were monitored in a hypereutrophic lake in an attempt to establish the contribution of phages to host mortality (Manage et al. 1999). Phage populations as high as 4.4×10^4 pfu mL^{-1} were detected and sharp decreases in the host population were correlated with increases in the phage population leading the authors conclude that phages may be a significant factor in bloom termination. Similarly high *M. aeruginosa* phage populations (5.6×10^4 pfu mL^{-1}) were reported for a sub-tropical Australian lake and a significant role in host mortality was proposed (Tucker and Pollard 2005). Viral mortality values as high as 52% of daily production of picocyanobacteria have been reported for a reservoir in the Volga-Baltic waterway (Kopylov et al. 2010). A different approach using real-time PCR to estimate phage abundance also revealed that the number of phages increased as host abundance declined, and that phage infection might cause shifts in the balance of microcystin-producing and non-producing *M. aeruginosa* strains (Yoshida et al. 2008a). Real-time PCR has also been used for the detection of cyanophage gene transcripts during infection of a netural *Microcystis aeruginosa* population in an attempt to assess the quantitative impacts of phage infection (Yoshida et al. 2010). Recently, it was shown that viruses (a siphovirus) from an Australian freshwater lake were able to lyse cells of *Cylindrospermopsis raciborskii* causing the filaments to split into smaller, but viable, fragments (Pollard and Young 2010).

The clearest evidence for the impact of phage infection on a freshwater cyanobacterial community comes from a series of studies on the shallow eutrophic Lake Loosdrecht in the Netherlands. During an experiment in two laboratory-scale enclosures (130 l each) in which the cyanobacterial community was composed primarily of *Limnothrix* sp. and *Prochlorothrix hollandica*, an almost complete lysis of the filamentous cyanobacterial population was observed (van Hannen et al. 1999). Electron microscopy revealed virus-like particles inside the cyanobacterial filaments and this observation taken together with a large increase in counts of extracellular virus-like particles this led to the conclusion that viral lysis had caused crash of the filamentous cyanobacterial population. This event was accompanied by large changes in the bacterial and eukaryotic communities. Further experiments were conducted in these laboratory scale enclosures to establish the conditions that might trigger the collapse of the filamentous cyanobacterial population (Gons et al. 2002). The predominating filamentous cyanobacteria grew for 2 weeks, but then the populations collapsed in both enclosures, whereas coccoid cyanobacteria persisted. The collapse again coincided with a peak in the counts of virus-like particles. Transmission electron microscopy showed phages with a myovirus morphology attached to and emerging from cyanobacterial cells. It was suggested that the rapid supply of nutrients might be a contributory factor to bloom collapse.

Dynamic modelling of the viral impact on cyanobacterial populations in shallow lakes indicated that burst sizes in the range 200–400 would be required for collapse of the host population (Gons et al. 2006). A recent study was aimed at studying the temporal variation and community assemblage of Lake Loosdrecht (Tijdens et al. 2008a). It had been recognized that the dominant, apparently homogeneous filamentous cyanobacterial in the lake was actually a diverse assemblage of cyanobacteria belonging to at least five different taxa belonging to the *Limnothrix*/*Pseudanabaena* group (Zwart et al. 2005). Viral abundances were found to be among the variables that best explained the cyanobacterial community assemblage, but no significant correlation between viral and cyanobacterial community was established (Tijdens et al. 2008a). However, estimates of the viral lysis contribution to filamentous cyanobacterial mortality during the period December 2004 and January 2005 indicated that between 84% and 97% of potential cyanobacterial production was removed by viral lysis (Tijdens et al. 2008b). By contrast microzooplankton grazing was estimated to remove between 90% and 99% of the potential unicellular cyanobacterial production.

21.4.1.2 Marine Cyanophage Studies

There have also been studies in the marine environment focussing on the impact of phage infection of unicellular phycoerythrin-containing *Synechococcus* subcluster 5.1 strains and there is also one report implicating phages in the decline of a coastal *Lyngbya majuscula* bloom (Hewson et al. 2001). *Synechococcus* phage abundance in Woods Hole Harbor ranged from 1.9×10^1 mL^{-1} in June to 1.14×10^4 mL^{-1} in July (Waterbury and Valois 1993) and in coastal waters of the Gulf of Mexico abundances ranging from undetectable to in excess of 10^5 mL^{-1} were reported (Suttle and Chan 1993; Suttle and Chan 1994). There is evidence of both seasonal (Waterbury and Valois 1993; Marston and Sallee 2003; Sandaa and Larsen 2006) and diel variations in *Synechococcus* phage abundance (Clokie et al. 2006a, b, c). The first study

aimed at assessing the impact of phages on marine *Synechococcus* populations employed a direct approach using electron microscopy to estimate that, depending on the sampling station, between 0.8% and 28% of cyanobacterial cells contained mature phages (Proctor and Fuhrman 1990). Assuming that the eclipse period was 90% of the latent period, they estimated that percentage of infected cells was actually tenfold greater than the observed frequency (i.e. 8–28%) and if there was a steady state where cell division was balanced by mortality, and if the latent period was roughly equal to the generation time, then the total mortality attributable to phages would be twice the number of infected cells at any given time (i.e. 16–56%). Several of these assumptions have been questioned. The eclipse period has been suggested to be more like 50% of the latent period (Waterbury and Valois 1993) and the idea that absolute mortality is twice the number of infected cells has been criticized by Binder (Binder 1999).

A different approach was aimed at attempting to assess the extent to which cells were resistant to the co-occurring cyanophages (Waterbury and Valois 1993). Ten clones of *Synechococcus* and seven phage isolates were obtained from a single water sample. The *Synechococcus* clones isolated from the 100- and 1,000-fold dilutions, which were assumed to be representative of the most abundant strains in the sample, were resistant to most of the phage isolates. In contrast, isolates from lower dilutions that were presumed to have outcompeted the resistant strains during enrichment exhibited sensitivity to more of the phage isolates. This trend was interpreted to mean that the majority of the *Synechococcus* population was resistant to the co-occurring phages. Three assumptions underlie this interpretation; (i) that the enrichment process did not select against abundant strains that grew slowly under laboratory conditions, (ii) that the sensitive strains had a growth advantage under laboratory conditions and (iii) that the phages isolated were truly representative of the abundant phages at the time of sampling rather than those that efficiently infected the hosts used for isolation. The coevolution of hosts and phage has also been studied using chemostat cultures of WH7803 and a model lytic phage. The authors showed that multiple co-evolutionary cycles occurred within a six month period which were concomitant with viral genotypic changes (Marcia et al. 2012).

In the natural environment phages are subjected to a variety of decay processes, particularly inactivation by sunlight and adsorption onto particles, and so to maintain a standing stock of virus net viral removal rates must be balanced by viral production. Thus, knowledge of viral decay rates can be used, in combination with assumptions regarding the burst size, to calculate the proportion of a host population that must be infected to maintain the population of phage. Using this approach it was calculated for waters off the coast of Texas that c. 6.5% of the *Synechococcus* cells would have to be lysed on a daily basis in order to maintain the phage population (Suttle and Chan 1994). A subsequent study (Garza and Suttle 1998) indicated that higher values might be more appropriate for the phage decay rates and a much lower value of the burst size was applied. The changes in the values of the decay rates and burst sizes largely compensated for each other and figures for the proportion of the *Synechococcus* community infected ranged from 1% to 8% for offshore waters, whereas in near shore waters only 0.01–0.02% of *Synechococcus* was lysed on a daily basis. Recent studies have extended these observations and shown that an accumulation of cyclobutane pyrimidine dimers (CPDs) formed in response to UV light within the cyanophage PP increased significantly with radiation intensity rather than exposure time (Liao et al. 2010). These experiments highlight the difficulties in estimating phage damage due to UV light and suggest that much more work needs to be done in this area.

21.4.2 Phage Infection Versus Protistan Grazing

Natural populations of unicellular cyanobacteria are potentially subject to two major biotic causes of mortality, namely phage infection and protistan grazing. The situation with filamentous and colonial forms may be more complex. Amoebic grazing of freshwater phycocyanin-rich *Synechococcus* strains has been described (Dillon and Parry 2009) and has also been shown to effect the genetic structure of a *Microcystis* bloom (Van Wichelen et al. 2010). Other studies have established planktonic flagellates and ciliates as major predators of freshwater and marine synechococci e.g. (Sigee et al. 1999; Chan et al. 2009; Zwirglmaier et al. 2009). Several attempts have been made to examine the relative contribution of these two processes to the dynamics of marine *Synechococcus* populations, though the methods used have an associated suite of assumptions and uncertainties. Several protists that graze on *Synechococcus* and the closely related *Prochlorococcus* have been identified (Caron et al. 1991; Strom 1991; Guillou et al. 2001; Christaki et al. 2002; Jeong et al. 2005). One study in an offshore site in the English channel detected no discernible viral lysis of the *Synechococcus* population and found that microzooplankton grazing was the major cause of mortality, but there were questions raised regarding the implications and limitations of the approach used (Kimmance et al. 2007). Similarly, grazing was found to dominate over viral lysis for *Synechococcus* in the deep chlorophyll maximum during the IRONAGES III study in the oligotrophic subtropical northeastern Atlantic (Baudoux et al. 2008). Again, grazing was found to be a more substantial cause of mortality than viral lysis in the majority of stations sampled during the summer in the North Sea (Baudoux et al. 2008). The constraints that determine whether virus- or grazer-mediated mortality dominates in marine or freshwater systems are not yet understood (Wilhelm and Matteson 2008).

The interaction between phage infection and protistan grazing may be more complicated than simple competition for a resource. There are several potential areas of interaction. Phage-infected cells may be more or less attractive to grazers than their otherwise isogenic relatives. This could apply to both temperate and obligately lytic phages. It has been suggested lysogeny may have been a key factor in the evolution of resistance to protistan grazing via the elaboration of toxins (Brussow 2007). Cell surface features that could include LPS, S-layer proteins and hydrophobicity are important, in addition to size, in prey selection by protists (Monger et al. 1999; Wildschutte et al. 2004; Tarao et al. 2009) and genetic diversity of freshwater *Synechococcus* (Ernst et al. 1996) and marine *Synechococcus* and *Prochlorococcus* (Sect. 21.4.1) have been associated with modification of surface structures. It has been shown that protistan grazers were selective in the preferences for different marine *Synechococcus* strains, and that some strains were ingested, but not digested (Zwirglmaier et al. 2009). The same study revealed a marked difference in grazing susceptibility between a spontaneous phage-resistant mutant and the wild type strain. Cyanophages have been shown to encode potential S-layer proteins (Mann et al. 2005) and LPS-associated enzymes (Sullivan et al. 2005) and studies (unpublished) in this laboratory have shown that grazers distinguish between phage-infected and uninfected cells. It may be that that ingestion may actually trigger the induction of prophages (Clarke 1998).

21.4.3 Genetic Diversity and Clonal Selection

The previous discussion has looked at the impact of phages on the whole *Synechococcus* population, but in fact there is a substantial literature detailing the genetic diversity of this group of organisms e.g. (Dufresne et al. 2008; Scanlan et al. 2009) and consequently the impact of phages may be to influence community composition. The distribution patterns of different clades of *Synechococcus* and *Prochlorococcus* are remarkably similar in different ocean systems with similar environmental conditions, but are markedly different in the four major ocean domains or biomes (Zwirglmaier et al. 2008). Very few studies have looked at temporal variation in genetic diversity. A 3 year time series looking at *Synechococcus* diversity in a coastal site in the northeastern Pacific Ocean showed that two clades dominated the community, but their relative abundance showed strong seasonal variations (Tai and Palenik 2009). Use of the *ntcA* gene to analyse *Synechococcus* and *Prochlorococcus* diversity in the oligotrophic waters of the Gulf of Aqaba revealed strong seasonal fluctuations in *Synechococcus* diversity in contrast to *Prochlorococcus* (Penno et al. 2006). Seasonal variations have also been detected in cyanophage populations (Marston and Sallee 2003; Sandaa and Larsen 2006). One study has examined the co-variation in the diversity of *Synechococcus* and cyanomyovirus communities to establish whether they were co-dependent (Mühling et al. 2005). In the Gulf of Aqaba a succession of *Synechococcus* genotypes was observed over an annual cycle. There were large changes in the genetic diversity of *Synechococcus,* which was reduced to one dominant genotype in July and both the abundance and genetic diversity of cyanophages covaried with that of *Synechococcus*. Multivariate statistical analyses showed a significant relationship between cyanophage assemblage structure and that of *Synechococcus*. These observations are consistent with cyanophage infection being a significant controlling factor in cyanobacterial clonal diversity and succession. Similarly, the composition of the population of cyanopodoviruses in Chesapeake Bay was found to exhibit distinct winter and summer patterns which were thought to be correlated with seasonal changes in the composition of the cyanobacterial population (Chen et al. 2009).

21.5 Lysogeny and Pseudolysogeny

A temperate phage may lyse an infected cell, but also has the potential to establish a stable relationship, lysogeny, with the infected cell by entering the prophage state which may be inherited by the progeny of the infected cell. Obligately lytic phages are genetically incapable of becoming prophages, but under certain conditions may not immediately cause lysis of the infected cell, a state or process known as pseudolysogeny. One of the more confusing areas of phage biology is that relating to these two phenomena of lysogeny and pseudolysogeny and the problem largely stems inconsistent use of terminology. This has been extensively discussed by Abedon (Abedon 2009) and the definitions that he proposes are those adopted here. Thus, a lysogenic strain is a strain in which every cell carries a prophage and perpetuates hereditarily the power to produce phage. Typically the prophage is integrated into the host cell chromosome, though in the case of some temperate phages the prophage may be maintained as an extrachromosomal element. Pseudolysogeny, by contrast, represents "a quiescent state following phage genome uptake during which that genome does not replicate, integration into the host chromosome does not occur, superinfection immunity is not expressed, and infection is not otherwise initiated". This state is temporary and may be resolved into lysis or lysogeny depending on the physiological status of the cell and environmental conditions.

21.5.1 Lysogeny

From early onwards in study of freshwater cyanophages a lysogenic relationship of some phages of filamentous cyanobacteria was established and has been extensively

reviewed (Sherman and Brown 1978; Martin and Benson 1988) and eventually *Plectonema boryanum* and phage LPP-2SP1 became the accepted system to study (Padan et al. 1972). However, much of this work was of a fundamental molecular biological nature and did not deal with the ecological significance of cyanophages. In most studies aimed at assessing the occurrence of lysogeny, chemical or physical agents, typically mitomycin C and UV light, are used to induce the prophage into the lytic cycle and the loss of intact cells or appearance of phage particles is measured. Unfortunately, defective prophages, bacteriocins and gene transfer agents may also be induced by these treatments and these treatments may not induce every prophage. Until recently there was virtually no information on lysogeny in unicellular cyanobacteria with the exception of a report suggesting that an *Anacystis nidulans* strain (*Synechococcus* PCC 6301) was lysogenic (Bisen et al. 1985) and that the prophage could be induced by copper ions (Lee et al. 2006) and another reporting the temperature-induced activation of the freshwater cyanophage AS-1 (Chu et al. 2011). In a study aimed at addressing an analysis of the prevalence of lysogeny in freshwater unicellular cyanobacteria, 19 strains of phycocyanin-rich *Synechococcus* were assessed for the induction of lysis following treatment with mitomycin C (Dillon and Parry 2008). Lysis of 16 out of the 19 strains was inducible with burst sizes in the range 3.5–23.7 phages per cell and electron microscopy revealed the phages to be siphoviruses. This one study suggests that lysogeny may be common in phycocyanin-rich *Synechococcus* population in freshwaters. It has been suggested recently that non-toxic cyclic peptides produced by bloom-forming cyanobacteria can cause lysis of *Microcystis aeruginosa* strains via the formation of virus-like particles (Sedmak et al. 2008), thereby implying a possible role for these peptides in the control of cyanobacterial population density. A strain of *M. aeruginosa* exposed to the depsipeptide planktopeptin BL1125 produced by *Planktothrix rubescens* was shown by electron microscopy to produce virus-like particles that corresponded in shape and size to podoviruses (Sedmak et al. 2009).

The potential occurrence of lysogeny in marine *Synechococcus* sub-cluster 5.1 strains has attracted much interest. In 2002 it was shown in a study employing mitomycin C induction and using water samples from Tampa Bay, Florida, that lysogeny occurred in natural *Synechococcus* populations and that there was a seasonal pattern (McDaniel et al. 2002). Induction occurred primarily during the late winter months and in keeping with theory may reflect low host abundance. Low host abundances are thought to select for temperate phages, whereas high abundances select for obligately lytic phages (Stewart and Levin 1984). This situation would apply when the host concentration falls below proliferation threshold and would be of the order of 10^4 hosts per mL (see Sect. 21.2.1). One induction event was also observed in late August which preceded a secondary Autumn bloom of *Synechococcus*. Lysogeny was also investigated during a bloom of *Synechococcus* in a pristine fjord in British Columbia, again employing mitomycin C induction (Ortmann et al. 2002). 0.6% of *Synechococcus* cells were inducible given typical estimates of bust sizes compared to 80% of heterotrophic bacteria. However, in both these studies the frequency of lysogeny must be a minimum estimate as a single *Synechococcus* host (WH7803) was used to titre cyanophages abundance. Subsequent studies have tended to confirm the correlation between low *Synechococcus* abundance and the frequency of lysogeny (McDaniel and Paul 2005; Long et al. 2008). Temperate phages may be favoured at low host abundances, but also under conditions of nutritional stress there is a tendency for temperate phages to lysogenize an infected host rather than enter the lytic cycle. The addition of nutrients (nitrate, ammonium, urea or phosphate) did not stimulate cyanophage production in response to mitomycin C in 8 out of 9 samples from Tampa Bay and the Gulf of Mexico (McDaniel and Paul 2005). Similarly the addition of phosphate was not found to have any effect on prophage induction (Williamson and Paul 2004). A group of 24 *Synechococcus* strains isolated from the Gulf of Mexico was screened for phage production in response to mitomycin C treatment and a significant increase in virus particles was found with 11 of the strains (46%) (McDaniel et al. 2006). One of the putative lysogens was induced by continuous high light and released phage particles with an apparently ssDNA genome. One study has been aimed at assessing the role of prophages in shaping the composition of assemblages (Hewson and Fuhrman 2007). The addition of mitomycin C to seawater had effects on bacterioplankton community structure and amongst the most negatively impacted OTUs were organisms classified as γ–Proteobacteria, SAR11 cluster and *Synechococcus*. There are other reports of lysogeny in marine cyanobacteria other than for *Synechococcus* subcluster 5.1 strains. The isolation of a temperate phage, ms-1, infecting a non-subcluster 5.1 marine *Synechococcus* strain has been described (Sode et al. 1994) as well as a temperate siphovirus infecting *Phormidium persicinum* (Ohki and Fujita 1996). The phenomenon of autoplaque formation which is the appearance of plaques within a bacterial lawn that has not been infected with phage and is thought to be due to the spontaneous induction of a prophage has been observed for about 50% of clonal *Nodularia spumigena* isolates from the Baltic Sea with either cyanomyoviruses or cyanosiphoviruses being present within the cell lysates; autoplaque formation was associated with senescent *Nodularia* cultures and cultures exposed to high light/temperature (C Jenkins and P.K. Hayes, personal communication, 2007). Trichomes of the marine diazotroph *Trichodesmium* release virus-like particles in response to treatment with mitomycin C (Ohki 1999; Hewson et al. 2004).

There is continuing interest in the role of copper ions as an inducing agent for temperate cyanophages. Copper appears to influence the distribution and abundance of marine phytoplankton in marine environments, and cyanobacteria are thought to be the most sensitive group to copper toxicity (Debelius et al. 2009) with coastal strains of marine *Synechococcus* species being more tolerant to copper shock than open-ocean strains (Stuart et al. 2009). The copper component of atmospheric aerosols has been implicated in toxic effects on marine *Synechococcus* (Paytan et al. 2009). Copper ions have been implicated in the induction of the marine temperate phage ms-1 (Sode et al. 1997) as well as the freshwater phage AS-1 (Lee et al. 2006).

It has already been mentioned that lysogeny may be an advantage to the phage when host abundance is low, but are there any selective benefits to the host? One might expect that the carriage of prophage DNA would impose a genetic burden on the host, however, the relationship between the prophage and its host is not as simple as that. Studies with phages of *E. coli* in fact show that prophage carriage can improve the fitness of the host in certain environments e.g. the *bor* and *lom* genes of phage λ are involved in serum resistance of the host cell and adhesion to human epithelial cells respectively (Barondess and Beckwith 1995; Pacheco et al. 1997). A prophage may significantly alter the phenotype of the host, a phenomenon known as phage conversion e.g. the gene for cholera toxin is carried by a prophage (Waldor and Mekalanos 1996). Furthermore, the lysogenized host will acquire immunity to super-infection by homoimmune phages. This implies a disadvantage for the non-lysogenic strain for in the natural environment the prophage will be induced in a proportion of the lysogenic hosts leading to the release of infectious phages, which will select against sensitive non-lysogenic closely related hosts. It has also been proposed that another potential benefit could be the silencing of host genes by phage repressors (Paul 2008). As yet there is no clear evidence of temperate cyanophages conferring any of these benefits on their hosts. However, the cyanosiphovirus P-SS2 that infects *Prochlorococcus* has been shown to be potentially capable of integration into its host genome by virtue of its possession of bioinformatically defined *int*, *bet* and *exo* genes together with a 53 bp attachment site (Sullivan et al. 2009). Also the "horned" cyanopodovirus Syn5 has a putative integrase gene (Pope et al. 2007) as does the freshwater cyanomyovirus Ma-LMM01 together with putative phage repressors (Yoshida et al. 2008b), which are suggestive of access to the temperate life cycle.

21.5.2 Pseudolysogeny

Pseudolysogeny is a quiescent state following phage genome introduction into the host during which the typical infection process is not initiated. This state is temporary and may be resolved into lysis or lysogeny depending on the physiological status of the cell and environmental conditions. It has been suggested that pseudolysogeny may be of central importance in considerations of community ecology (Abedon 2009). There is some evidence that obligately lytic *Synechococcus* phages can enter the pseudolysogenic state (Wilson et al. 1996). When *Synechococcus* sp. WH7803 cells were grown in phosphate-replete or phosphate-deplete media and infected with the obligately lytic phage S-PM2 (a myovirus) there was an apparent 80% reduction in the burst size under phosphate-deplete conditions. However, this apparent reduction in the burst size actually reflected the fact that 100% of the phosphate-replete cells lysed, compared to only 9% of the phosphate-deplete cells, suggesting that the majority of phosphate-deplete cells were pseudolysogens i.e. the phages had adsorbed and introduced their genomes into the host, but were not undergoing the typical infection process. The temporary pseudolysogenic state and its resolution into lysis was confirmed by the re-addition of phosphate. Similar observations were made with two other obligately lytic *Synechococcus* myoviruses, S-WHM1 and S-BM1. Experiments with natural assemblages in Tampa Bay and the Gulf of Mexico provided some indirect evidence of pseudolysogeny, when the addition of phosphate caused an increase in lytic phage production whilst having no effect on prophage induction (McDaniel and Paul 2005). Temperate phages can alter the phenotype of their lysogenic host via the phenomenon of phage conversion and in the pseudolysogenic state both lytic and temperate phages might be able modify the phenotype of the host cell thus bringing about what might be termed "pseudolysogenic conversion" (Mann 2006). It remains to be established what phage genes, if any, are being expressed in the pseudolysogenic state. As yet there is no information regarding cyanophages and very little from other phage:host systems.

21.6 Phage Sensitivity, Resistance and Phenotypic Variability

There are three aspects of the resistance of potential hosts to phage infection that are important to consider in terms of the impact of phages on natural populations. These include the apparent resistance by some strains to phages that will infect closely related strains, the acquisition of immunity to infection by mutation or horizontal gene transfer and non-genetic individuality in genetically uniform populations. It is a familiar concept that strains of an individual bacterial species may exhibit differential sensitivities to infection by phages. Such differences can arise through diverse mechanisms including the presence or absence of phage receptors, phage exclusion systems affecting adsorption or DNA injection, restriction modification systems, receptor masking etc. (Hoskisson and Smith 2007; Labrie et al. 2010). Similarly the idea of an

arms race between phages and bacteria with hosts mutating to resistance and phages mutating to overcome resistance is well established from laboratory studies of communities of phages and their hosts (for review see (Bohannan and Lenski 1997)). Far less obvious is the idea that isogenic bacteria even in homogeneous environments may exhibit a range of phenotypes including interactions with phages (Pearl et al. 2008).

21.6.1 Strain-Specific Intrinsic Resistance

Studies on both freshwater and marine cyanobacteria have produced ample evidence that closely related strains of the same species may exhibit different patterns of sensitivity to phage infection (see Sects. 21.2.2 and 21.2.5). The specific receptors for phage attachment are commonly protein or lipopolysaccharide (LPS) components of the cell surface and the presence or absence of the receptors is likely to be a central component of this variability. The genetic variability between very closely related, but phenotypically distinct, strains of *Prochlorococcus* was found to occur mostly in genomic islands that encode, amongst other things, proteins involved in cell surface modification, including the biosynthesis of lipopolysaccharide and consequently may generate diversity in phage receptors (Coleman et al. 2006). Some of these island genes appear to have been acquired in part by phage-mediated horizontal gene transfer. Further genomic analysis of *Prochlorococcus* strains has revealed that the greatest differentiator between the most closely related isolates are genes related to outer membrane synthesis and cell surface structures are potentially under strongly diversifying selection if they serve as attachment or recognition sites for phages (Kettler et al. 2007). Similar observations relating to potential cell surface modifying glycosyl transferases have been made for *Synechococcus* sub-cluster 5.1 strains (Dufresne et al. 2008) and it has been suggested certain giant ORFs that are relatively widely dispersed in the marine *Synechococcus* genomes may also be involved in cell surface modification to prevent phage infection (Scanlan et al. 2009).

Restriction-modification systems are commonly responsible for such differences and certainly a large number of restriction-modification systems have been characterised in cyanobacteria e.g. (Zhao et al. 2006). Indeed phages themselves may encode restriction modification systems. The restriction enzyme *Eco*R1 was found to digest the DNA of four phages propagated on the *Synechococcus* subcluster 5.1 strain WH7803, but failed to digest the DNA of a fifth, the myovirus S-WHM1, suggesting that it encoded its own restriction-modification system (Wilson et al. 1993). Restriction can be invoked to explain the difference in the efficiency of plating of phage N-1 on *Anabaena* sp. PCC 7120 and *Anabaena variabilis* (Currier and Wolk 1979).

Another potential reason for differential efficiency of infection between host strains is immunity arising from lysogeny. Lysogenic cells are resistant to superinfection by the same temperate phage or secondary infection by homoimmune phages. Some lytic phages are also able to prevent secondary infection by other lytic or temperate phages by a process known as superinfection exclusion (Labrie et al. 2010).

21.6.2 Acquisition of Resistance by Mutation or Horizontal Gene Transfer

Growth of sensitive bacteria in the presence of phages rapidly selects for the appearance of mutants that have developed resistance to the co-occurring phages(s). As has been mentioned previously phage receptors are commonly protein or LPS components of the cell surface and mutations leading to phage resistance usually alter such cell surface structures. Lipopolysaccharide has been implicated in the absorption of the cyanomyovirus AS-1, by virtue of the ability of purified polysaccharide to inactivate AS-1 (Samimi and Drews 1978). The O antigen of LPS is comprised of serially repeated, strain-specific oligosaccharide units and disruption in *Anabaena* sp. strain PCC 7120 of the genes predicted to encode undecaprenyl-phosphate galactosephosphotransferase (*rfbP*) and mannosyl transferase (*rfbZ*), enzymes involved in the O antigen synthesis, led to resistance to obligately lytic phage A-1(L) and to the temperate phage A-4(L) (Xu et al. 1997). Electrophoretic analysis showed that the interruption of the *rfbP* and *rfbZ* genes led to a change in or loss of the characteristic pattern length of the LPS. Analysis of spontaneous phage-resistant *Synechococcus* sub-cluster 5.1 mutants has also implicated LPS as an important phage receptor (this laboratory – unpublished results). Phage N-1 did not adsorb to phage-resistant mutants of *Nostoc muscorum*, but host range mutants of N-1 could isolated that were capable of adsorption with varying efficiencies (Sarma and Kaur 1997). Mutants *Anabaena* sp. Strain PCC 7120 resistant to phage AN-15 could be isolated by prolonged incubation (Mole et al. 1997). Two classes of resistant cells were identified; those in which the phage did not adsorb and those where adsorbtion occurred, but subsequent replication was defective. Again it proved possible to isolate mutants of AN-15 that were able to infect the previously phage-resistant host mutants.

Commonly mutation to phage resistance comes with a fitness cost (Bohannan and Lenski 2000) with phage-resistant bacteria usually being less efficient competitors for growth-limiting resources than their sensitive ancestors (Buckling and Rainey 2002). The fitness cost of mutation to phage resistance has been investigated in *Synechococcus* subcluster 5.1 strains. Four such strains and 32 cyanophages were used in combination to select for phage-resistant

mutants 23 of which were selected for further study (Stoddard et al. 2007). Measurements of adsorption kinetics suggested that resistance was due to changes in phage receptors and cross-resistance was commonly observed in the mutants. The growth rates of these mutants was measured to test for a cost of resistance (COR) (Lennon et al. 2007). A detectable COR was only found in 50% of cases and when observed represented a ~20% reduction in relative fitness compared to the ancestral strains. However, there was a much clearer relationship between COR and compositional resistance i.e. easily detectable fitness costs were associated with mutation to resistance to particular phages.

A genomic analysis of 77 mutants of strains of *Prochloroccus* selected for resistance to any of ten phages revealed that the mutations occurred primarily in non-conserved, horizontally transferred genes that localized to a single hypervariable genomic island (Avrani et al. 2011).The mutations effected viral receptors on the surface of cells, but also imposed fitness costs of either reduced growth rate or enhanced sensitivity to infection by other phages.These results are evidence that viral receptors are primarily located in genomic islands and that phages represent a potent selection pressure on both island content and island gene bdiversity.

One of the most interesting recent developments in our understanding of resistance to phage infection has been the discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) and the related *cas* (CRISPR-associated) genes. Together these repeats and genes constitute an unique defence system that leads to acquired immunity that operates by interfering with phage proliferation via small non-coding RNAs and can be passed on to subsequent generations (for review, see van der Oost et al. 2009). There is evidence for CRISPRs to be involved in the evolution of phage resistance in cyanobacteria in microbial mat communities (Heidelberg et al. 2009). Genomic data were obtained from two thermophilic *Synechococcus* isolates from microbial mats in hot springs in Yellowstone National Park. Two distinct CRISPR types were found in both *Synechococcus* genomes and a third was found in one of the genomes that was shared with other organisms from the mat. Although this study provided clear evidence for the importance and adaptive nature of CRISPRs in a mat community, there is little evidence from the many sequenced cyanobacterial genomes that CRISPRs are widespread in cyanobacteria.

21.6.3 Phenotypic Variability

Isogenic bacteria can exhibit a range of phenotypes (Gefen and Balaban 2009) and such non-genetic individuality can extend to phage-host interactions. Furthermore, the nature of the cell surface is likely to be influenced by environmental factors, such as the presence or absence of specific proteins involved in nutrient transport. It was shown as early as 1940 for a coliphage, that the adsorption rate constant under optimal growth conditions was more than 60 times greater than under poor conditions (Delbrück 1940). Consequently both the presence and density of some receptors might be influenced by nutrient availability. A study of phage λ and *Escherichia coli* showed that persister bacteria (drug-sensitive cells that can persist under antibiotic selection), which exist in a state of dormancy or slow growth, are protected from prophage induction, but not from lytic infection (Pearl et al. 2008). It has been reported in the case of a coastal strain of *Synechococcus* that phage would only bind to about 10% of the cells, suggesting that only a small proportion of the population were expressing the particular phage receptor at any particular time (Suttle 2000). Light has also been shown to affect phage adsorption to cyanobacterial hosts. Studies on phage S-PM2 infecting the *Synechococcus* subcluster 5.1 strain WH7803 revealed a striking dependence on light for adsorption and this was also observed with a purified cell envelope fraction (Jia et al. 2010). Such a marked light dependence of adsorption has not been observed with freshwater cyanobacteria, although some degree of light dependence for adsorption has been reported for the cyanomyovirus AS-1 (Cseke and Farkas 1979; Kao et al. 2005) and phage progeny production was correlated with the amount of light in the laboratory and occurred in a diel pattern under natural light (Kao et al. 2005).

21.7 Molecular Taxonomy of Cyanophages

There is a currently an unresolved debate concerning phage taxonomy, though there is a general trend way from phonetic approaches to classification towards molecular taxonomic analyses, particularly as more and more phage genomes are sequenced e.g. (Lavigne et al. 2009). Molecular taxonomy is the designation of phage taxa according to their molecular signature. In practice relationships are reconstructed by phylogenetic analysis of aligned gene or protein sequences. There are several different methods of performing these analysis and the three most common are based on (a) neighbour joining (where similarity is used to group taxa), (b) parsimony (which looks for shared characters between two or more taxa and assumes this means they share a common ancestry) and maximum likelihood (which examines all possible relationships) these approaches are explained well in (Hall 2004). These approaches are a really powerful tool for examining evolutionary relationships within bacteriophages. Where bacteriophages and cyanobacteria have genes in common it can also be used to establish relationships between them and their phages. Although extremely useful, it is important to add a word of caution from the outset. Molecular taxonomy has moved from being a specialist discipline

practiced by taxonomists to far more general usage. A 'true' phylogeny must be based on a rigorous alignment, the application of several different algorithms, suitable statistical support and an interpretation of the phylogeny based on known caveats of the technique. Sadly these procedures are often not followed and some published cyanophage phylogenies have consequently been over interpreted.

Bacteria (including cyanobacteria) have universal taxonomic markers which are used in molecular taxonomy, the most widely used of which is the 16S subunit of the ribosomal RNA. However, unlike the situation for bacteria, there is no universal molecular marker for bacteriophages as the three families of bacteriophages do not share significant conservation of any one gene (Paul et al. 2002). There are, however, apparently 'universal' cyanophage family primers, in particular for the myoviruses, which are the most commonly isolated family of cyanophages and many phage diversity studies have relied solely on the use of these molecular markers as such an approach has the advantage of speed and also that viruses which cannot be isolated can still be identified. Various genes have been used to study the molecular diversity of myoviruses; Fuller was the first to design a cyanomyovirus-specific primer to amplify a portion of the gene that encodes the phage portal protein (gp20) (Fuller et al. 1998). This gene encodes the protein at the top of the neck of the phage through which the DNA passes en route through the tail sheath. The gene seems conserved enough to be universal among cyanomyoviruses, but gives a useful phylogenetic resolution with which to examine viral diversity. These primers have been used and modified by several researchers e.g. (Zhong et al. 2002; Marston and Sallee 2003; Dorigo et al. 2004; Sullivan et al. 2008; Wang et al. 2009a) to try to establish the true diversity of marine and fresh water cyanomyoviruses both from isolated cyanophages and directly from water samples. These studies collectively suggest that the marine cyanophages are in phylogenetic groups distinct from fresh water cyanophages (Dorigo et al. 2004). Samples from the floodwater of a Japanese paddy field have also formed distinct groups but these are more closely related to the sequences from the freshwater environment than those from the marine environment (Wang et al. 2009a; 2011). However, whilst g20 may be a reasonable tool to analyse diversity it is not a good predictor of a cyanophage's host or habitat (Sullivan et al. 2008). Another phage gene that has been used to analyse myovirus diversity is that encoding the major capsid structural protein gp23 (Filee et al. 2005). Although these primers appear to show the existence of new cyanophage groups, their specificity is uncertain as they may also amplify gp23 from heterotrophic T4-like phages. The DNA polymerase gene has also been used to study marine cyanopodovirus diversity (Huang et al. 2010).

The *psbA* gene encodes a core component of photosystem II and its occurrence in a phage genome was established for the cyanomyovirus S-PM2 and it has now been shown to be present present in the majority of cyanomyoviruses (Mann et al. 2003; Lindell et al. 2004; Millard et al. 2004; Sullivan et al. 2006), but is also found in podoviruses (Sullivan et al. 2006; Wang and Chen 2008) and in freshwater cyanophages (Chenard and Suttle 2008; Wang et al. 2009b). Consequently, *psbA* has been proposed as an additional genetic marker that can be used to explore the diversity and evolutionary history of cyanophages in aquatic environments (Zeidner et al. 2005; Chenard and Suttle 2008). More recently several *psbA* phylogenies have been published (Chenard and Suttle 2008). Although the presence of *psbA* in cyanophages is of enormous ecological relevance, the gene it is itself a poor taxonomic marker. Although it has been shown to be useful for resolving relationships between some plant groups such as red algae (Broom et al. 2008), it is a highly conserved protein and therefore it seems to suffer from homoplasies (where the same mutation has arisen independently), which confuses attempts to analyse phylogenetic relationships This, is particularly true with respect to the low-light *Prochlorococcus* which has very few DNA repair mechanisms (Coleman et al. 2006). Point mutations and homologous recombination, are both involved in the generation of microdiversity between closely related phages, both for core phage genes like g20 and 'host-derived' genes such as *psbA*, and although much of this diversity is neutral some amino acid substitutions are observed which have the potential to influence the population dynamics of phage-host interactions (Marston and Amrich 2009).

21.8 Isolation of Cyanophages

Techniques involved in the isolation of phages from the environment have been well reviewed Carlson (Carlson 2005). The conventional method of phage isolation is by plaque formation whereby a lawn of the appropriate host is grown in a comparatively dilute top layer of agar lying on top of a more concentrated layer of agar containing required nutrients. This technique is variously referred to as the top layer, soft agar overlay, double overlay or double agar layer method. Not all phages will form visible plaques in top agar lawns and other approaches are needed for their isolation. Similarly, not every host is amenable to growth as a lawn in agar and in this situation isolation in liquid culture via the most probable number method is appropriate. If viruses are not present in sufficiently high abundance in the environment a concentration or enrichment stage is necessary. The viral fraction in the can be concentrated using tangential flow filtration, polyethylene glycol precipitation, or sequential adsorption and elution. the viruses in the water can be concentrated by enrichment on a host prior to a plaque assay. Different phages yield plaques with different morphologies varying in features such as plaque size,

turbidity and nature of the plaque edge and these aspects of plaque formation are discussed by Abedon and Yin (Abedon and Yin 2008).The methods for the isolation of cyanophages are in principle the same as those for any other phages and have been reviewed in the case of aquatic environments by Millard (Millard 2009).

To isolate phages using plaque assays requires the phages to be suited to making plaques on lawns of cyanobacteria and their hosts to be able to grow as lawns. This approach was widely adopted in the early studies aimed at the isolation of phages infecting freshwater cyanobacteria and can be used with both obligately lytic and temperate cyanophages. More recently, this approach was widely applied to the isolation of phages infecting marine sub-cluster 5.1 *Synechococcus* strains. To isolate phages using this method requires a permissive host and for *Synechococcus* phages the strain *Synechococcus* WH7803 is commonly used (Marston and Sallee 2003; Millard and Mann 2006) (Fig. 21.2). This strain of *Synechococcus* was isolated from the Sargasso Sea from mesotrophic water and is considered to be a generalists in terms of its physiology (Scanlan et al. 2009) and is sensitive to infection by phages from a wide variety of geographical provinces including the Red Sea, the Atlantic Ocean and the Pacific Ocean e.g. (Suttle and Chan 1993; Waterbury and Valois 1993; Millard and Mann 2006). It is generally not necessary to concentrate sea water first in order to isolate bacteriophages in this way and often in oligotrophic waters one can obtain up to 200 phage plaques from 100 µl sea water (Marston and Sallee 2003; Clokie et al. 2006a, b, c).

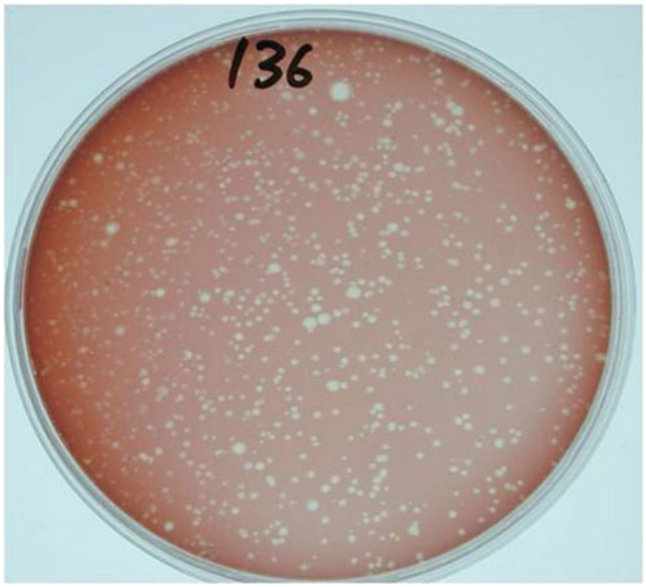

Fig. 21.2 Lawn of the marine cyanobacterium *Synechococcus* WH7803 with phage plaques from a seawater sample clearly visible. Note the variations in plaque size and turbidity

Temperate cyanophages occurring as free phage particles can yield plaques in top layers, but where temperate phages are to be isolated from lysogenic hosts, prophage induction via chemical or physical agents such as Mitomycin C or UV light must be employed prior to plating and thought must be given to a suitable host to be used in the lawn as the original lysogen will not yield plaques. Before being characterised, bacteriophages are usually purified from plaque assays by being put through at least three rounds of purification where plaques are picked and used to infect fresh lawns. It is known that some phages are lost during this process and one such group in particular which are lost are what are thought to be marine temperate cyanophages (Fig. 21.3). Sometimes, during the initial direct isolation of cyanophages, instead of obtaining clear plaques with discrete edges (a), one isolates turbid plaques which have cells growing in the middle of the plaque and the plaque itself may exhibit a ragged appearance and (b, c). These putative temperate phages appear to be inherently unstable and can not be further plaque propagated (unpublished data from MRJC and A.D. Millard, personal communication 2007).

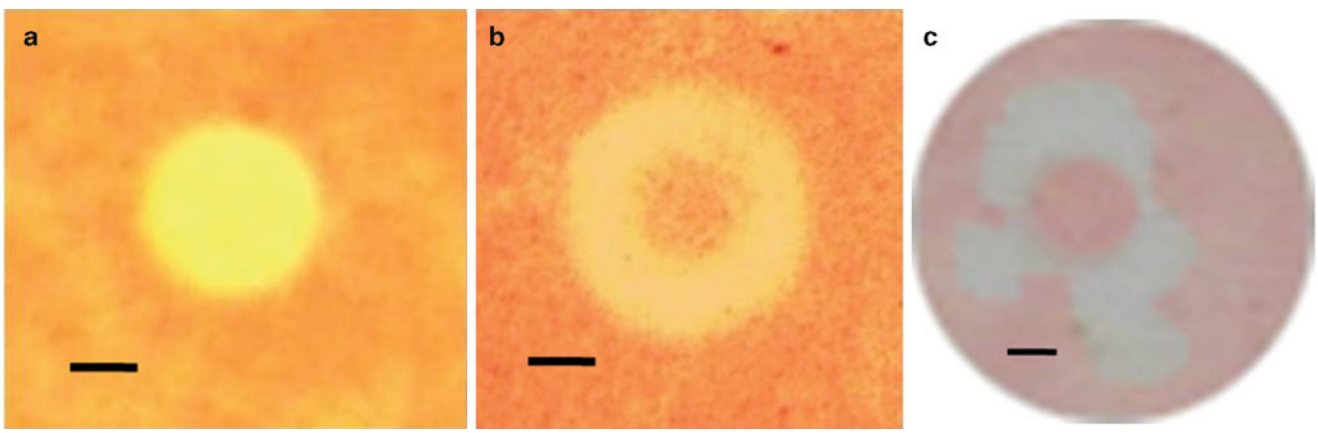

Fig. 21.3 Comparison of obligately lytic (**a**) and putatively temperate (**b** and **c**) marine cyanophage plaques. The temperate phage plaques may have smooth (**b**) or crenate (**c**) edges. Scale bar is 1 mm

Some cyanobacterial genera such as the ubiquitous marine *Prochlorococcus* cannot be propagated on plates and therefore to isolate bacteriophages sea water is added to cultures growing in the wells of microtitre dishes. Other cyanobacteria are highly motile on plates, which is incompatible with plaque formation, and also require that phage isolation be done in liquid culture e.g. *Planktothrix* strains (Deng and Hayes 2008). In essence the procedure involves the preparation of a dilution series of the phage sample such that the highest dilution is not expected to contain phage (Carlson 2005). The host is grown in an appropriate medium and aliquots transferred to the wells of a microtitre dish in which aliquots of the phage dilution series. After incubation for an appropriate length of time the wells that exhibited culture lysis at the highest phage dilution are considered to have contained a single phage. Usually several rounds of this procedure are carried out to ensure the clonality of the phage.

21.9 Cyanophage Genomics and Insights into Ecophysiology of Infection

By 2010 24 genomes had been sequenced and published of phages that infect marine *Synechococcus*; P60, S-PM2, Syn-5, S-RSM4 (Chen and Lu 2002; Mann et al. 2005; Pope et al. 2007; Millard et al. 2009) S-SM1, S-SSM5, S-SSM7, S-SM2, S-ShM2, SYN1, SYN33, SYN19 (Sullivan et al. 2010), and *Prochlorococcus*; P-SSM2, PSSM4, P-SS2, P-SSM7, P-RSM4, P-HM2, P-HM1 (Sullivan et al. 2005, 2009, 2010), and one, Syn9, that infects both *Synechococcus* and *Prochlorococcus* (Weigele et al. 2007). The remaining three infect *Microcystis aeruginosa*; Ma-LMM01 and *Phormidium foveolarum*; Pf-WMP4 and Pf-WMP3 (Liu et al. 2007; Liu et al. 2008; Yoshida et al. 2008a, b). Most of these viruses are myoviruses, but Syn5 is a non-standard podovirus by virtue of its horned capsid appearance, Pf-WMP4, Pf-WMP3, P60 and P-SSP7 are podoviruses and P-SS2 is a siphovirus. A comparison of 26 T4-like myoviral genomes including 16 marine cyanomyoviruses revealed a conserved "core" of a 38 virion structure and DNA replication genes and a cyanomyovirus "core" set of 25 genes that included six previously described genes with putative functions (*psbA, mazG, phoH, hsp20, hli03, cobS*), a hypothetical protein with a potential phytanoyl-CoA dioxygenase domain, two virion structural genes, and 16 hypothetical genes (Sullivan et al. 2010). There is currently a large drive to sequence many more cyanophage genomes. This is due to an appreciation of their complexity and importance and facilitated by the reduced cost of sequencing and the improvements in the technology available to conduct high throughput sequencing of phage genomes. This represents a major research effort and will hopefully reveal further insights into the biology of cyanophages.

The marine cyanomyoviruses so far isolated are clearly related to the archetypal myovirus T4 in terms of phage structural genes and synteny and comparative analysis of their genomes has offered tantalising insights into their evolution and their close relationship with their hosts (Millard et al. 2009). In the 16 sequenced cyanobacteriophage genomes it appears that there is a highly core set of 32 genes present in all genomes this includes structural genes and what look like host derived genes. Several of these phage-acquired host genes appear to have a role in photosynthesis and nutrient acquisition and regulation (both for phosphorous and nitrogen). This has revealed novel features of the phage-host interaction with respect to their physiology as described below. Acquisition of these genes appears to be associated with a hyperplastic region within the conserved structural gene module (Millard et al. 2009). These data have been significantly extended by a recent analysis of the marine T4-Like phage scaffolds from the GOS metagenome (Comeau et al. 2010). These data suggest that the sequenced cultured representatives of cyanophages are true representatives of viruses that exist in nature. They highlight the importance of cyanophages in their hosts evolution as clearly 'host' genes can evolve at a much greater rate when not confined to the constrains of a cyanobacterial genome.

Unlike the situation in the marine cyanobacteria, *Microcystis* cyanomyovirus Ma-LMM01 possesses very few genes with homologues to known phage genes and therefore the authors propose it belongs to a new subgroup within the Myoviridae family (Yoshida et al. 2008a, b). Of the 28 ORFs that the authors could be assigned function to 13 appear to be involved in DNA processing and nucleotide metabolism. Only one gene exhibits recognizable homology with a host gene and that is *nblA* which is thought to be involved in phycobilisome degradation.

The podoviruses that have been sequenced thus far are all part of the T7-like podophage supergroup (Chen and Lu 2002; Sullivan et al. 2005; Liu et al. 2007; Liu et al. 2008). As is the case with marine myoviruses they have recognisable structural genes which are also conserved in gene order. The genomes are relatively small and consist of around 45 genes. Pf-WMP4 and Pf-WMP3 (*Phormidium foveolarum*) have diverged from all other podoviruses at the DNA level, but are closely related at protein level and in terms of genome architecture. The marine siphovirus P-SS2 is larger and considerably divergent from the previously sequenced siphoviruses (Sullivan et al. 2009), though it appears to be most closely related to the lambdoid siphoviruses with which it shares 13 functional homologues. It lacks the photosynthesis-associated gene *psbA*, but does contain 14 other cyanobacterial homologues. Two further siphoviruses have recently been sequenced which infect the marine cyanobacterium *Acaryochloris marina*. These phages have relatively small genomes of 55 and 57 kb, again most of the genes had no

homologues amongst known genes, but they did contain several genes of interest including a gene that appears to be closely related to a eukaryotic DNA polymerase (Chan et al., submitted).

21.9.1 Effect on Host Physiology and the Roles of 'Host-Derived' Genes

Phages are parasites and rely entirely up on their host cell for reproduction. The infection process in the lytic pathway involves a major transformation of the infected cell from a metabolism geared towards optimal survival and reproduction to one directed to replication of the phage genome, morphogenesis of the virion, packaging and finally lysis. Two distinct strategies have been recognized for cyanophages by which the translation of phage transcripts may be enhanced (Limor-Waisberg et al. 2011) T7-like cyanopodoviruses adjust their GC content and codon usage to those of the host. T4-like cyanomyoviruses tend to have low GC content genomes, but encode their own tRNAs that are consonant with the preferred low GC codons. This may potentially broaden the potential host range of these myoviruses. Phage-encoded early proteins may target a wide variety of processes in the host cell that are not only important to optimizing phage reproduction, but also may be aimed at ensuring survival of the infected cell until lysis e.g. preventing protistan grazing (Sect. 21.2.4) or superinfection. The ability of prophages to alter the phenotype of the host cell via lysogenic conversion is a well recognized phenomenon, but alterations in the phenotype during lytic infection and/or pseudolysogeny are far less characterized. Many aspects of the potential ability of cyanophages to modulate the metabolism of their hosts is revealed from cyanophage genome analysis. This is particularly true of the marine cyanomyoviruses and to a lesser extent for the podoviruses and it remains to be seen whether it holds true for freshwater cyanophages as the number of sequenced genomes increases. Metagenomic studies confirm individual genome analysis with the frequency and genetic-linkage of phage-encoded (but host-derived) genes confirming their widespread distribution in natural viral populations and their probable functional importance to cyanophage replication (DeLong et al. 2006; Williamson et al. 2008). A good example is the *mazG* gene, which is almost universally present and conserved in marine cyanomyoviruses yet within this sequences from geographically diverse locations are quite divergent from each other (Bryan et al. 2008; Millard et al. 2009). MazG has been shown to prevent the normal accumulation of guanosine 3′,5′-bispyrophosphate (ppGpp) during the stringent response to amino acid starvation (Gross et al. 2006). ppGpp acts as a global regulator in *E. coli*, causing a redirection of transcription in favour of genes important for starvation survival (Magnusson et al. 2005), and has been shown to control elongation during DNA replication in response to nutritional status (Wang et al. 2007). In freshwater unicellular cyanobacteria ppGpp was demonstrated to accumulate under conditions of energy limitation (Mann et al. 1975) and nitrogen starvation (Friga et al. 1981), whereas phage infection interfered with this accumulation (Borbely et al. 1980). It has been suggested (Clokie and Mann 2006) that the phage-encoded MazG reduces the ppGpp pool in the infected cell thereby mimicking the physiological state of a cell replete with nutrients and thus optimizing phage reproduction by reactivating the pathways of macromolecular synthesis.

21.9.2 Photosynthetic Physiology

Marine cyanophages, particularly cyanomyoviruses and podoviruses, seem to have acquired several genes associated with photosynthesis, which they express on infection of their cyanobacterial host to retain the cell's photosynthetic apparatus throughout the infection cycle. These genes include *psbA* and *psbD* which encode D1 and D2, the main two proteins at the heart of the photocentre in photosystem II (Mann et al. 2003; Lindell et al. 2004; Sullivan et al. 2005; Millard et al. 2009). Recently, sequencing of the genome of a freshwater cyanomyovirus has revealed the presence of five of the six "signature genes" of the photosynthetic marine cyanomyoviruses, including *psbA* (Dreher et al. 2011). The maximum photosynthetic efficiency of the cells is undiminished by infection (Clokie et al. 2006a, b, c), whilst at the same time the D1 transcripts encoded by the host are replaced by those encoded by the phage (Lindell et al. 2005; Clokie et al. 2006a, b, c). An exciting twist to the regulation of *psbA* was recently suggested by Millard et al., who showed that a small phage-encoded antisense RNA may regulate its expression (Millard et al. 2010). Modelling work has also provided support to the hypothesis that these phage-encoded *psbA* and *psbD* genes increase the fitness of the bacteriophages especially under very intense light conditions (Bragg and Chisholm 2008; Hellweger 2009).

Evidence has now been obtained for the presence of photosystem 1 gene cassettes in marine virus genomes (Sharon et al. 2009; Alperovitch-Lavy et al. 2011) and it was proposed that the viral-PS1 components might provide an unique mechanism for transferring reducing power from respiratory and other electron transfer chains to PS1. However, the PS1 cassettes have not been found in any of the sequenced genomes and it remains to be seen how common they are in cyanophages. Amongst the other phage-encoded genes that are widespread in cyanomyoviruses and might play a role in modulating the photosynthetic physiology of the cell are *ho1*(haem oxygenase), *pcyA* (phycocyanobilin:ferredoxin oxidoreductase), *pebS* (phycoerythrobilin synthase), *petE*

(plastocyanin), *petF* (ferredoxin), *ptoX* (electron transfer to oxygen), *cpeT* (putative regulator of phycoerythrin biosynthesis), and *hli* (high-light inducible protein) (Lindell et al. 2004; Mann et al. 2005; Sullivan et al. 2005; Sullivan et al. 2006; Millard et al. 2009). It has been shown for four of these genes (*ho1*, *pebA*, *petF* and *pcyA*) that they mimic the activities of the corresponding host-encoded proteins (Dammeyer et al. 2008), though in the case of PebA it catalyses a reaction in phycoerythrin biosynthesis that requires two host-encoded enzymes. The *pebS* gene together with *petF* and *ho1* were found to be transcribed during infection suggesting that they enhance phage fitness. Shan and colleagues showed that phycoerythrin (and chlorophyll) per cell increased in *Synechococcus* sp. WH7803 cells infected by phage S-PM2 and produced evidence that the increase in host light-harvesting capacity was being regulated by CpeT (Shan et al. 2008). It has been suggested that petE, encoding plastocyanin, functions simply to maintain host photosynthetic electron transport (Millard et al. 2009). The *ptoX* gene is particularly interesting, as PTOX has a proposed role as an enzyme mediating alternative electron transfer upstream of PS1 using oxygen as a terminal electron acceptor (Bailey et al. 2008) and may function to prevent photo-inhibition. The *speD* gene found in S-PM2 (Mann et al. 2005) and P-SSM4 (Sullivan et al. 2005) encodes S-adenosylmethionine decarboxylase, a key enzyme in the biosynthesis of the polyamines, which have been suggested to play a role in the stability of *psbA*-2 mRNA (Mulo et al. 1998). It is also known in higher plants that polyamines influence the structure and oxygen evolution rate of the PSII reaction centre (Bograh et al. 1997). Thus these cyanophages may be increasing the stability of *psbA*-2 mRNA or of PSII itself as an additional mechanism to prolong the functional integrity the photosystem during the infection process.

21.9.3 Carbon Metabolism and Nutrient Acquisition

Cyanobacterial respiratory metabolism is dominated by the oxidative pentose phosphate pathway (OPP) and consequently it is intriguing that genes encoding key enzymes of the pathway have been found in marine phage genomes. The genes encoding transaldolase (*talC*), glucose-6-phosphate dehydrogenase (*zwf*) and 6-phosphogluconate dehydrogenase (*gnd*) are widespread in cyanomyoviruses (Lindell et al. 2004; Mann et al. 2005; Sullivan et al. 2005; Weigele et al. 2007; Millard et al. 2009). It has been proposed that the expression of OPP genes during phage infection might reflect a fitness benefit gained from access to reducing power from stored carbon substrates. It is also possible that the non-oxidative branch of the pentose phosphate pathway produces ribose-5-phosphate required for the synthesis of nucleotide precursors of DNA (Clokie and Mann 2006). In this context it is worth noting that the cyanomyovirus-encoded *cobS* gene encodes a key enzyme in cobalamin biosynthesis and this is a known cofactor of ribonucleotide reductase. The gene encoding a homologue of CP12, a Calvin cycle inhibitor, has now been identified in marine cyanophage genomes (Thompson et al. 2011). CP12 directs carbon flux into the pentose phosphate pathway and is further evidence for its proposed role in the supply of nucleotide precursors for phage DNA replication.

Phages in general have a high requirement for phosphate and but this nutrient is present at uM levels in the oligotrophic regions of the ocean where it limits cyanobacterial growth. One host-derived gene that seems to be abundant within marine cyanophages is *pstS*, which encodes a phosphate-binding protein that may increase the acquisition of phosphate during the infection cycle and appears to be widespread in cyanophage genomes (Millard et al. 2009). This gene has been shown to be common in viral metagenomic data sets, where it appears to be correlated with salinity and depth (Williamson et al. 2008), as has another cyanomyovirus-encoded phosphate-starvation-associated gene *phoH* Additional evidence for the role of these genes in phage infection comes from observations that the transcription of *pstS* is up-regulated in phage infected *Prochlorococcus* cells which are phosphate limited (Zeng and Chisholm 2012).

There is also evidence to suggest that phages influence nitrogen acquisition by manipulating their acquisition of ammonium by interfering with levels of the 2-oxoglutarate carbon skeleton which determine the activity of ammonia uptake (Sullivan et al. 2010).

21.10 Conclusions

Cyanophage biology started in the 1960s and was an important area of research for some 20 years, but then declined, in part because of the lack of success in the use of cyanophages to control nuisance blooms. The reawakening of interest was triggered by the discovery that phages in general are extremely abundant in aquatic systems and are likely to contribute to or affect major ecological processes. Substantial progress has been made in the isolation and characterization of cyanophages and this has been coupled with a growing appreciation of the importance, complexity and subtlety of their interactions with their hosts. This has been partially been due to the large number of sequenced cyanophage genomes revealing their content to contain a significant proportion of what is traditionally seen as host genes. The function of a significant number of these genes is yet to be determined and future work will reveal their structure and function which will in turn shed light on their biology. There are four main related areas of cyanophage biology

that not only require much further attention, but are likely to throw up novel features of the phage-host relationship and the contribution of phages to ecological processes. The first is the assessment of the impact of phages on the dynamics and diversity of natural cyanobacterial populations where we only have essentially preliminary evidence. The same may be said of the second important area for cyanophage biology, namely the interaction between phage infection and grazing. Perhaps the most exciting area is likely to be the way in which the cyanophages can modify the phenotype of the infected host and this will have important ramifications for both the interaction with grazers and population complexity and dynamics. Finally the contribution of cyanophages to the long-term evolution of their hosts needs to be established as we begin to unravel how cyanobacterial genes evolve within their host genomes. It may be that we have to look at cyanophages, not purely as parasites on their hosts, but as the very vectors that allow adaptation to a changing environment.

References

Abedon ST (2009) Disambiguating bacteriophage pseudolysogeny. In: Adams HT (ed) Contemporary trends in bacteriophage research. Nova Science Publishers, Inc, New York, pp 285–307, 447 pp

Abedon ST, Yin J (2008) Impact of spatial structure on phage population growth. In: Abedon ST (ed) Bacteriophage ecology: population growth, evolution, and impact of bacterial viruses. Cambridge University Press, Cambridge, UK, pp 94–112, 508 pp

Ackermann HW (2005) Bacteriophage classification. In: Kutter E, Sulakvelidze A (eds) Bacteriophages: biology and applications. CRC Press, Boca Raton, pp 67–89, 510 pp

Ackermann HW (2007) 5500 Phages examined in the electron microscope. Arch Virol 152:227–242

Ackermann HW, DuBow MS (1987) Bacteriophage taxonomy. In: Ackermann HW, DuBow MS (eds) Viruses of prokaryotes, vol 1. CRC Press, Boca Raton, pp 13–44, 480 pp

Alperovitch-Lavy A, Sharon I, Rohwer F, Aro E-M, Glaser F, Milo R, Nelson N, Beja O (2011) Reconstructing a puzzle: existence of cyanophages containing both photosystem I and photosystem II gene suites inferred from oceanic metagenomic datasets. Environ Microbiol 13:24–32

Avrani S, Wurtzel O, Sharon I, Sorek R, Lindell D (2011) Genomic island variability facilitates *Prochlorococcus*-virus coexistence. Nature 474:604–608

Bailey S, Melis A, Mackey KRM, Cardol P, Finazzi G, van Dijken G, Berg GM, Arrigo K, Shrager J, Grossman A (2008) Alternative photosynthetic electron flow to oxygen in marine *Synechococcus*. Biochim Biophys Acta Bioenerg 1777:269–276

Barondess JJ, Beckwith J (1995) Bor gene of phage Lambda, involved in serum resistance, encodes a widely conserved outer-membrane lipoprotein. J Bacteriol 177:1247–1252

Baudoux AC, Veldhuis MJW, Noordeloos AAM, van Noort G, Brussaard CPD (2008) Estimates of virus- vs. grazing induced mortality of picophytoplankton in the North Sea during summer. Aquat Microb Ecol 52:69–82

Bergh O, Borsheim KY, Bratbak G, Heldal M (1989) High abundance of viruses found in aquatic environments. Nature 340(6233):467–468

Binder B (1999) Reconsidering the relationship between virally induced bacterial mortality and frequency of infected cells. Aquat Microb Ecol 18:207–215

Bisen PS, Audholia S, Bhatnagar AK (1985) Mutation to resistance for virus AS-1 in the cyanobacterium *Anacystis nidulans*. Microbios 29:7–12

Bograh A, Gingras Y, TajmirRiahi HA, Carpentier R (1997) The effects of spermine and spermidine on the structure of photosystem II proteins in relation to inhibition of electron transport. FEBS Lett 402:41–44

Bohannan BJM, Lenski RE (1997) Effect of resource enrichment on a chemostat community of bacteria and bacteriophage. Ecology 78:2303–2315

Bohannan BJM, Lenski RE (2000) Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. Ecol Lett 3:362–377

Borbely G, Kari C, Gulyas A, Farkas GL (1980) Bacteriophage infection interferes with guanosine 3′-diphosphate-5′-diphosphate accumulation induced by energy and nitrogen starvation in the cyanobacterium *Anacystis nidulans*. J Bacteriol 144:859–864

Bragg JG, Chisholm SW (2008) Modeling the fitness consequences of a cyanophage-encoded photosynthesis gene. PLoS One 3:e3550

Broom JES, Hart DR, Farr TJ, Nelson WA, Neill KF, Harvey AS, Woelkerling WJ (2008) Utility of *psbA* and nSSU for phylogenetic reconstruction in the Corallinales based on New Zealand taxa. Mol Phylogenet Evol 46:958–972

Brussow H (2007) Bacteria between protists and phages: from antipredation strategies to the evolution of pathogenicity. Mol Microbiol 65:583–589

Brussow H, Kutter E (2005) Phage ecology. In: Kutter E, Sulakvelidze A (eds) Bacteriophages: biology and applications. CRC Press, Boca Raton, pp 129–162, 510 pp

Bryan MJ, Burroughs NJ, Spence EM, Clokie MRJ, Mann NH, Bryan SJ (2008) Evidence for the intense exchange of *mazG* in marine cyanophages by horizontal gene transfer. PLoS One 3:e2048

Buckling A, Rainey PB (2002) Antagonistic coevolution between a bacterium and a bacteriophage. Proc R Soc Biol Sci Ser B 269:931–936

Cannon RE, Shane MS, Demichel E (1974) Ecology of blue-green algal viruses. J Environ Eng Div 100:1205–1211

Carlson K (2005) Working with bacteriophages: common techniques and methodological approaches. In: Kutter E, Sulakvelidze A (eds) Bacteriophages: biology and applications. CRC Press, Boca Raton, pp 437–494, 510 pp

Caron DA, Lim EL, Miceli G, Waterbury JB, Valois FW (1991) Grazing and utilization of chroococcoid cyanobacteria and heterotrphic bacteria by protozoa in laboratory cultures and a coastal plankton community. Mar Ecol Prog Ser 76:205–217

Chan YF, Tsai AY, Chiang KP, Hsieh CH (2009) Pigmented nanoflagellates grazing on *Synechococcus*: seasonal variations and effect of flagellate size in the coastal ecosystem of subtropical Western Pacific. Microb Ecol 58:548–557

Chen F, Lu J (2002) Genomic sequence and evolution of marine cyanophage P60: a new insight on lytic and lysogenic phages. Appl Environ Microbiol 68:2589–2594

Chen F, Wang K, Huang S, Cai H, Zhao M, Jiao N, Wommack KE (2009) Diverse and dynamic populations of cyanobacterial podoviruses in the Chesapeake Bay unveiled through DNA polymerase gene sequences. Environ Microbiol 11:2884–2892

Chenard C, Suttle CA (2008) Phylogenetic diversity of sequences of cyanophage photosynthetic gene *psbA* in marine and freshwaters. Appl Environ Microbiol 74:5317–5324

Chisholm SW, Olson RJ, Zettler ER, Goericke R, Waterbury JB, Welschmeyer NA (1988) A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature 334:340–342

Christaki U, Courties C, Karayanni H, Giannakourou A, Maravelias C, Kormas KA, Lebaron P (2002) Dynamic characteristics of *Prochlorococcus* and *Synechococcus* consumption by bacterivorous nanoflagellates. Microb Ecol 43:341–352

Chu T-C, Murray SR, Hsu S-F, Vega Q, Lee LH (2011) Temperature-induced activation of freshwater cyanophage AS-1 prophage. Acta Histochem 113:294–299

Clarke KJ (1998) Virus particle production in lysogenic bacteria exposed to protozoan grazing. FEMS Microbiol Lett 166:177–180

Clokie MRJ, Mann NH (2006) Marine cyanophages and light. Environ Microbiol 8:2074–2082

Clokie MRJ, Millard AD, Mehta JY, Mann NH (2006a) Virus isolation studies suggest short-term variations in abundance in natural cyanophage populations of the Indian Ocean. J Mar Biol Assoc UK 86:499–505

Clokie MRJ, Shan JY, Bailey S, Jia Y, Krisch HM, West S, Mann NH (2006b) Transcription of a 'photosynthetic' T4-type phage during infection of a marine cyanobacterium. Environ Microbiol 8:827–835

Clokie MRJ, Millard AD, Metha JY, Mann NH (2006c) Virus isolation studies suggest diel cycles in viral abundance in natural cyanophage populations of the Indian Ocean. J Mar Biol Assoc UK 86:499–505

Coleman M, Sullivan MB, Martiny AC, Steglich C, Barry K, DeLong EF, Chisholm SW (2006) Genomic islands and the ecology of *Prochlorococcus*. Science 311:1768–1770

Comeau AM, Arbiol C, Krisch HM (2010) Gene network visualization and quantitative synteny analysis of more than 300 marine T4-Like phage scaffolds from the GOS metagenome. Mol Biol Evol 27:1935–1944

Coulombe AM, Robinson GGC (1981) Collapsing *Aphanizomenon flos-aquae* blooms: possible contributions of photo-oxidation, O_2 toxicity, and cyanophages. Can J Bot 59:1277–1284

Cseke CS, Farkas GL (1979) Effect of light on the attachment of cyanophage AS-1 to *Anacystis nidulans*. J Bacteriol 137:667–669

Currier TC, Wolk CP (1979) Characteristics of *Anabaena variabilis* influencing plaque formation by cyanophage N-1. J Bacteriol 139:88–92

Dammeyer T, Bagby SC, Sullivan MB, Chisholm SW, Frankenberg-Dinkel N (2008) Efficient phage-mediated pigment biosynthesis in oceanic cyanabacteria. Curr Biol 18:442–448

Debelius B, Forja JM, DelValls TA, Lubian LM (2009) Toxicity of copper in natural marine picoplankton populations. Ecotoxicology 18:1095–1102

Delbrück M (1940) Adsorption of bacteriophage under various physiological conditions of the host. J Gen Physiol 23:631–642

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard N-U, Martinez A, Sullivan MB, Edwards R, Brito BR, Chisholm SW, Karl DM (2006) Community genomics among stratified microbial assemblages in the ocean's interior. Science 311:496–502

Deng L, Hayes PK (2008) Evidence for cyanophages active against bloom-forming freshwater cyanobacteria. Freshw Biol 53: 1240–1252

Desjardins PR, Olsen GB (1983) Viral control of nuisance cyanobacteria (blue-green algae). California Water Resources Center Contribution No. 185. California Water Resources Center, University of California, Davis, pp 1–35

Desnues C, Rodriguez-Brito B, Rayhawk S, Kelley S, Tran T, Haynes M, Liu H, Furlan M, Wegley L, Chau B, Ruan YJ, Hall D, Angly FE, Edwards RA, Li LL, Thurber RV, Reid RP, Siefert J, Souza V, Valentine DL, Swan BK, Breitbart M, Rohwer F (2008) Biodiversity and biogeography of phages in modern stromatolites and thrombolites. Nature 452:340–342

Dillon A, Parry JD (2008) Characterization of temperate cyanophages active against freshwater phycocyanin-rich *Synechococcus* species. Freshw Biol 53:1253–1261

Dillon A, Parry JD (2009) Amoebic grazing of freshwater *Synechococcus* strains rich in phycocyanin. FEMS Microbiol Ecol 69:106–112

Dorigo U, Jacquet S, Humbert JF (2004) Cyanophage diversity, inferred from g20 gene analyses, in the largest natural lake in France, Lake Bourget. Appl Environ Microbiol 70:1017–1022

Dreher TW, Brown N, Bozarth CS, Schwartz AD, Riscoe E, Thrash C, Bennett SE, Tzeng S-C, Maier CS (2011) A freshwater cyanophage whose genome indicates close relationships tophotosynthetic marine cyanomyophages. Environ Microbiol 13:1858–1874

Dufresne A, Ostrowski M, Scanlan DJ, Garczarek L, Mazard S, Palenik BP, Paulsen IT, Tandeau de Marsac N, Wincker P, Dossat C, Ferriera S, Johnson J, Post AF, Hess WR, Partensky F (2008) Unraveling the genomic mosaic of a ubiquitous genus of marine cyanobacteria. Genome Biol 9:R90

Ernst A, Postius C, Böger P (1996) Glycosylated surface proteins reflect genetic diversity among *Synechococcus* species of Lake Constance. In: Simon M, Güde H, Weisse T (eds) Aquatic microbial ecology. Proceedings of the 6th international workshop on the measurement of microbial activities in the cycling of matter in aquatic environments, Konstanz, Germany, 17–22 April 1995. Advances in limnology (Ergebnisse Limnologie), vol 48. Schweizerbart, Stuttgart, pp 1–6, 276 pp

Fallon RD, Brock TD (1979) Lytic organisms and photooxidative effects: influence on blue-green algae (cyanobacteria) in Lake Mendota, Wisconsin. Appl Environ Microbiol 38:499–505

Filee J, Tetart F, Suttle CA, Krisch HM (2005) Marine T4-type bacteriophages, a ubiquitous component of the dark matter of the biosphere. Proc Natl Acad Sci USA 102:12471–12476

Friga GM, Borbely G, Farkas GL (1981) Accumulation of guanosine tetraphosphate (ppGpp) under nitrogen starvation conditions in *Anacystis nidulans*, a cyanobacterium. Arch Microbiol 129:341–342

Fuller NJ, Wilson WH, Joint IR, Mann NH (1998) Occurrence of a sequence in marine cyanophages similar to that of T4 g20 and its application to PCR-based detection and quantification techniques. Appl Environ Microbiol 64:2051–2060

Garza DR, Suttle CA (1998) The effect of cyanophages on the mortality of *Synechococcus* spp. and selection for UV-resistant viral communities. Microb Ecol 36:281–292

Gefen O, Balaban NQ (2009) The importance of being persistent: heterogeneity of bacterial populations under antibiotic stress. FEMS Microbiol Rev 33:704–717

Ghai R, Martin-Cuadrado A-B, Molto AG, Heredia IG, Cabrera R, Martin J, Verdu M, Deschamps P, Moreira D, Lopez-Garcia P, Mira A, Rodriguez-Valera F (2010) Metagenome of the Mediterranean deep chlorophyll maximum studied by direct and fosmid library 454 pyrosequencing. ISME J 4:1154–1166

Gons HJ, Ebert J, Hoogveld HL, van den Hove L, Pel R, Takkenberg W, Woldringh CJ (2002) Observations on cyanobacterial population collapse in eutrophic lake water. Antonie Van Leeuwenhoek 81:319–326

Gons HJ, Hoogveld HL, Simis SGH, Tijdens M (2006) Dynamic modelling of viral impact on cyanobacterial populations in shallow lakes: implications of burst size. J Mar Biol Assoc UK 86:537–542

Gross M, Marianovsky I, Glaser G (2006) MazG – a regulator of programmed cell death in *Escherichia coli*. Mol Microbiol 59:590–601

Guillou L, Jacquet S, Chretiennot-Dinet MJ, Vaulot D (2001) Grazing impact of two small heterotrophic flagellates on *Prochlorococcus* and *Synechococcus*. Aquat Microb Ecol 26:201–207

Guttman B, Raya P, Kutter E (2005) Basic phage biology. In: Kutter E, Sulakvelidze A (eds) Bacteriophages: biology and applications. CRC Press, Boca Raton, pp 29–66, 510 pp

Guyader S, Burch CL (2008) Optimal foraging predicts the ecology but not the evolution of host specialization in bacteriophages. PLoS One 3:e1946

Hall BG (2004) Phylogenetic trees made easy: a how-to manual. Sinauer Associates, Inc, Sunderland

Heidelberg JF, Nelson WC, Schoenfeld T, Bhaya D (2009) Germ warfare in a microbial mat community: CRISPRs provide insights into the co-evolution of host and viral genomes. PLoS One 4:e4169

Hellweger FL (2009) Carrying photosynthesis genes increases ecological fitness of cyanophage *in silico*. Environ Microbiol 11:1386–1394

Hennes KP, Suttle CA, Chan AM (1995) Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. Appl Environ Microbiol 61:3623–3627

Hewson I, Fuhrman JA (2007) Characterization of lysogens in bacterioplankton assemblages of the Southern California Borderland. Microb Ecol 53:631–638

Hewson I, O'Neil JM, Dennison WC (2001) Virus-like particles associated with *Lyngbya majuscula* (Cyanophyta; Oscillatoriaceae) bloom decline in Moreton Bay, Australia. Aquat Microb Ecol 25:207–212

Hewson I, Govil SR, Capone DG, Carpenter EJ, Fuhrman JA (2004) Evidence of *Trichodesmium* viral lysis and potential significance for biogeochemical cycling in the oligotrophic ocean. Aquat Microb Ecol 36:1–8

Hoskisson PA, Smith MCM (2007) Hypervariation and phase variation in the bacteriophage 'resistome'. Curr Opin Microbiol 10:396–400

Huang S, Wilhelm SW, Jiao N, Chen F (2010) Ubiquitous cyanobacterial podoviruses in the global oceans unveiled through viral DNA polymerase gene sequences. ISME J 4:1243–1251

Jenkins CA, Hayes PK (2006) Diversity of cyanophages infecting the heterocystous filamentous cyanobacterium *Nodularia* isolated from the brackish Baltic Sea. J Mar Biol Assoc UK 86:529–536

Jeong HJ, Park JY, Nho JH, Park MO, Ha JH, Seong KA, Jeng C, Seong CN, Lee KY, Yih WH (2005) Feeding by red-tide dinoflagellates on the cyanobacterium *Synechococcus*. Aquat Microb Ecol 41:131–142

Jia Y, Shan J, Millard A, Clokie MR, Mann NH (2010) Light-dependent adsorption of photosynthetic cyanophages to *Synechococcus* sp. WH7802. FEMS Microbiol Lett 310:120–126

Johnson DW, Potts M (1985) Host range of LPP cyanophages. Int J Syst Bacteriol 35:76–78

Johnson PW, Sieburth JM (1979) Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. Limnol Oceanogr 24:928–935

Kao CC, Green S, Stein B, Golden SS (2005) Diel infection of a cyanobacterium by a contractile bacteriophage. Appl Environ Microbiol 71:4276–4279

Kasman LM, Kasman A, Westwater C, Dolan J, Schmidt MG, Norris JS (2002) Overcoming the phage replication threshold: a mathematical model with implications for phage therapy. J Virol 76:5557–5564

Kettler GC, Martiny AC, Huang K, Zucker J, Coleman ML, Rodrigue S, Chen F, Lapidus A, Ferriera S, Johnson J, Steglich C, Church GM, Richardson P, Chisholm SW (2007) Patterns and implications of gene gain and loss in the evolution of *Prochlorococcus*. PLoS Genet 3:2515–2528

Kimmance SA, Wilson WH, Archer SD (2007) Modified dilution technique to estimate viral versus grazing mortality of phytoplankton: limitations associated with method sensitivity in natural waters. Aquat Microb Ecol 49:207–222

Kopylov AI, Kosolapov DB, Zabotkina EA, Straskrabova V (2010) Distribution of picocyanobacteria and virioplankton in mesotrophic and eutrophic reservoirs: the role of viruses in mortality of picocyanobacteria. Izv Akad Nauk Ser Biol/Rossiiskaia Akad Nauk 6:661–669

Labrie SJ, Samson JE, Moineau S (2010) Bacteriophage resistance mechanisms. Nat Rev Microbiol 8:317–327

Lavigne R, Darius P, Summer EJ, Seto D, Mahadevan P, Nilsson AS, Ackermann HW, Kropinski AM (2009) Classification of *Myoviridae* bacteriophages using protein sequence similarity. BMC Microbiol 9:224

Lawrence JG, Hatfull GF, Hendrix RW (2002) Imbroglios of viral taxonomy: genetic exchange and failings of phenetic approaches. J Bacteriol 184:4891–4905

Lee LH, Lui D, Platner PJ, Hsu SF, Chu TC, Gaynor JJ, Vega QC, Lustigman BK (2006) Induction of temperate cyanophage AS-1 by heavy metal-copper. BMC Microbiol 6:17

Lennon JT, Khatana SAM, Marston MF, Martiny JBH (2007) Is there a cost of virus resistance in marine cyanobacteria? ISME J 1:300–312

Levin BR, Bull JJ (2004) Population and evolutionary dynamics of phage therapy. Nat Rev Microbiol 2:166–172

Liao MJ, Cheng K, Yang JY, Zhao YJ, Shi ZL (2010) Assessment of UV-B damage in cyanophage PP. Aquat Microb Ecol 58:323–328

Limor-Waisberg K, Carmi A, Scherz A, Pilpel Y, Furman I (2011) Specialization versus adaptation: two strategies employed by cyanophages to enhance their translation efficiencies. Nucleic Acids Res 39:6016–6028

Lindell D, Sullivan MB, Johnson ZI, Tolonen AC, Rohwer F, Chisholm SW (2004) Transfer of photosynthesis genes to and from *Prochlorococcus* viruses. Proc Natl Acad Sci USA 101:11013–11018

Lindell D, Jaffe JD, Johnson ZI, Church GM, Chisholm SW (2005) Photosynthesis genes in marine viruses yield proteins during host infection. Nature 438:86–89

Liu XY, Shi M, Kong SL, Gao Y, An CC (2007) Cyanophage Pf-WMP4, a T7-like phage infecting the freshwater cyanobacterium *Phormidium foveolarum*: complete genome sequence and DNA translocation. Virology 366:28–39

Liu XY, Kong SL, Shi M, Fu LW, Gao Y, An CC (2008) Genomic analysis of freshwater cyanophage Pf-WMP3 infecting yanobacterium *Phormidium foveolarum*: the conserved elements for a phage. Microb Ecol 56:671–680

Long A, McDaniel LD, Mobberley J, Paul JH (2008) Comparison of lysogeny (prophage induction) in heterotrophic bacterial and *Synechococcus* populations in the Gulf of Mexico and Mississippi river plume. ISME J 2:132–144

Magnusson LU, Farewell A, Nystrom T (2005) ppGpp a global regulator in Escherichia coli. Trends Microbiol 13:236–242

Manage PM, Kawabata Z, Nakano S (1999) Seasonal changes in densities of cyanophage infectious to *Microcystis aeruginosa* in a hypereutrophic pond. Hydrobiologia 411:211–216

Mann NH (2003) Phages of the marine cyanobacterial picophytoplankton. FEMS Microbiol Rev 27:17–34

Mann NH (2006) Phages of cyanobacteria. In: Calendar R (ed) The bacteriophages. Oxford University Press, New York, pp 517–532, 746 pp

Mann NH, Carr NG, Midgley JEM (1975) RNA synthesis and accumulation of guanine nucleotides during growth shift down in the blue-green alga *Anacystis nidulans*. Biochim Biophys Acta 402:41–50

Mann NH, Cook A, Millard A, Bailey S, Clokie M (2003) Marine ecosystems: bacterial photosynthesis genes in a virus. Nature 424:741

Mann NH, Clokie MRJ, Millard A, Cook A, Wilson WH, Wheatley PJ, Letarov A, Krisch HM (2005) The genome of S-PM2, a "photosynthetic" T4-type bacteriophage that infects marine *Synechococcus* strains. J Bacteriol 187:3188–3200

Marcia F, Marstona FJ, Pierciey Jra, Alicia S, Gary G, Ji Q, Chandri Y, Stephan CS, Matthew RH, Jennifer BHM (2012) 1 Rapid diversification of coevolving marine *Synechococcus* and a virus 109(12):4544–4549

Marhaver KL, Edwards RA, Rohwer F (2008) Viral communities associated with healthy and bleaching corals. Environ Microbiol 10:2277–2286

Marston MF, Amrich CG (2009) Recombination and microdiversity in coastal marine cyanophages. Environ Microbiol 11:2893–2902

Marston MF, Sallee JL (2003) Genetic diversity and temporal variation in the cyanophage community infecting marine *Synechococcus* species in Rhode Island's coastal waters. Appl Environ Microbiol 69:4639–4647

Martin EL (1982) The biological regulation of bloom-causing blue-green algae: a feasible alternative. Nebraska Water Resources Center Project completion report A-056, pp 1–36

Martin E, Benson R (1988) Phages of cyanobacteria. In: Calendar R (ed) The bacteriophages, vol 2. Plenum Press, New York, pp 607–645

Martin EL, Leach JE, Kuo KJ (1978) The biological regulation of bloom-causing blue-green algae. In: Louitit MW, Miles JAR (eds) Microbial ecology. Springer, Berlin, pp 62–67

McDaniel L, Paul JH (2005) Effect of nutrient addition and environmental factors on prophage induction in natural populations of marine *Synechococcus* species. Appl Environ Microbiol 71:842–850

McDaniel L, Houchin LA, Williamson SJ, Paul JH (2002) Plankton blooms – Lysogeny in marine *Synechococcus*. Nature 415:496

McDaniel LD, delaRosa M, Paul JH (2006) Temperate and lytic cyanophages from the Gulf of Mexico. J Mar Biol Assoc UK 86:517–527

Millard AD (2009) Isolation of cyanophages from aquatic environments. In: Clokie MRJ, Kropinski AM (eds) Bacteriophages: methods and protocols, vol 1. Humana Press, New York, pp 33–42, 307 pp

Millard AD, Mann NH (2006) A temporal and spatial investigation of cyanophage abundance in the Gulf of Aqaba, Red Sea. J Mar Biol Assoc UK 86:507–515

Millard A, Clokie MRJ, Shub DA, Mann NH (2004) Genetic organization of the *psbAD* region in phages infecting marine *Synechococcus* strains. Proc Natl Acad Sci USA 101:11007–11012

Millard AD, Zwirglmaier K, Downey MJ, Mann NH, Scanlan DJ (2009) Comparative genomics of marine cyanomyoviruses reveals the widespread occurrence of *Synechococcus* host genes localized to a hyperplastic region: implications for mechanisms of cyanophage evolution. Environ Microbiol 11:2370–2387

Millard AD, Gierga G, Clokie MRJ, Evans DJ, Hess WR, Scanlan DJ (2010) An antisense RNA in a lytic cyanophage links *psbA* to a gene encoding a homing endonuclease. ISME J 4:1121–1135

Mole R, Meredith D, Adams DG (1997) Growth and phage resistance of *Anabaena* sp. strain PCC 7120 in the presence of cyanophage AN-15. J Appl Phycol 9:339–345

Monger BC, Landry MR, Brown SL (1999) Feeding selection of heterotrophic marine nanoflagellates based on the surface hydrophobicity of their picoplankton prey. Limnol Oceanogr 44:1917–1927

Mühling M, Fuller NJ, Millard A, Somerfield PJ, Marie D, Wilson WH, Scanlan DJ, Post AF, Joint I, Mann NH (2005) Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton. Environ Microbiol 7:499–508

Mulo P, Eloranta T, Aro EM, Maenpaa P (1998) Disruption of a *spe*-like open reading frame alters polyamine content and *psbA*-2 mRNA stability in the cyanobacterium *Synechocystis* sp. PCC 6802. Bot Acta 111:71–76

Murray AG, Jackson AJ (1992) Viral dynamics: a model of the effects of size, shape, motion and abundance of single-celled planktonic organisms and other particles. Mar Ecol Prog Ser 89:103–116

Ohki K (1999) A possible role of temperate phage in the regulation of *Trichodesmium* biomass. Bull d l'Inst Oceanogr Monaco 19:287–291

Ohki K, Fujita Y (1996) Occurrence of a temperate cyanophage lysogenizing the marine cyanophyte *Phormidium persicinum*. J Phycol 32:365–370

Ortmann AC, Lawrence JE, Suttle CA (2002) Lysogeny and lytic viral production during a bloom of the cyanobacterium *Synechococcus* spp. Microb Ecol 43:225–231

Pacheco SV, Gonzalez OG, Contreras GLP (1997) The lom gene of bacteriophage lambda is involved in *Escherichia coli* K12 adhesion to human buccal epithelial cells. FEMS Microbiol Lett 156:129–132

Padan E, Shilo M (1969) Distribution of cyanophages in natural habitats. Verh Int Verein Limnol 17:747–751

Padan E, Shilo M (1973) Cyanophages – viruses attacking blue-green algae. Bacteriol Rev 37:343–370

Padan E, Shilo M, Oppenheim AB (1972) Lysogeny of the blue-green alga *Plectonema boryanum* by LPP2-SPI cyanophage. Virology 47:525–526

Paul JH (2008) Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? ISME J 2:579–589

Paul JH, Sullivan MB, Segall AM, Rohwer F (2002) Marine phage genomics. Comp Biochem Physiol B Biochem Mol Biol 133:463–476

Payne RJH, Jansen VAA (2000) Phage therapy: the peculiar kinetics of self-replicating pharmaceuticals. Clin Pharmacol Ther 68:225–230

Payne RJH, Jansen VAA (2002) Evidence for a phage proliferation threshold? J Virol 76:13123–13124

Paytan A, Mackey KRM, Chen Y, Lima ID, Doney SC, Mahowald N, Labiosa R, Postf AF (2009) Toxicity of atmospheric aerosols on marine phytoplankton. Proc Natl Acad Sci USA 106:4601–4605

Pearl S, Gabay C, Kishony R, Oppenheim A, Balaban NQ (2008) Nongenetic individuality in the host-phage interaction. PLoS Biol 6:957–964

Pedulla ML, Ford ME, Houtz JM, Karthikeyan T, Wadsworth C, Lewis JA, Jacobs-Sera D, Falbo J, Gross J, Pannunzio NR, Brucker W, Kumar V, Kandasamy J, Keenan L, Bardarov S, Kriakov J, Lawrence JG, Jacobs WR, Hendrix RW, Hatfull GF (2003) Origins of highly mosaic mycobacteriophage genomes. Cell 113:171–182

Penno S, Lindell D, Post AF (2006) Diversity of *Synechococcus* and *Prochlorococcus* populations determined from DNA sequences of the N-regulatory gene *ntcA*. Environ Microbiol 8:1200–1211

Philips EJ, Monegue RL, Aldridge FJ (1990) Cynophages which impact bloom-forming cyanobacteria. J Aquat Plant Manage 28:92–97

Pollard PC, Young LM (2010) Lake viruses lyse cyanobacteria, *Cylindrospermopsis raciborskii*, enhances filamentous-host dispersal in Australia. Acta Oecol 36:114–119

Pope WH, Weigele PR, Chang J, Pedulla ML, Ford ME, Houtz JM, Jiang W, ChiuW HGF, Hendrix RW, King J (2007) Genome sequence, structural proteins, and capsid organization of the cyanophage Syn5: a "horned" bacteriophage of marine *Synechococcus*. J Mol Biol 368:966–981

Proctor LM, Fuhrman JA (1990) Viral mortality of marine bacteria and cyanobacteria. Nature 343:60–62

Rodda KM (1996) Temporal and spatial dynamics of *Synechococcus* spp. and *Micromonas pusilla* host-viral systems. Marine Science, University of Texas at Austin, Austin

Rodriguez-Valera F, Martin-Cuadrado A-B, Rodriguez-Brito B, Pasic L, Thingstad TF, Rohwer F, Mira A (2009) Explaining microbial population genomics through phage predation. Nat Rev Microbiol 7:828–836

Rohwer F, Edwards R (2002) The phage proteomic tree: a genome-based taxonomy for phage. J Bacteriol 184:4529–4535

Rohwer F, Prangishvili D, Lindell D (2009) Roles of viruses in the environment. Environ Microbiol 11:2771–2774

Safferman RS (1973) Phycoviruses. In: Carr NG, Whitton BA (eds) The biology of blue-green algae. Blackwell Scientific Publications, Oxford, pp 214–237, 676 pp

Safferman RS, Morris ME (1963) Algal virus isolation. Science 140:679–680

Safferman RS, Morris ME (1967) Observations on the occurrence, distribution, and seasonal incidence of blue-green algal viruses. Appl Microbiol 15:1219–1222

Samimi B, Drews G (1978) Adsorption of cyanophage AS-1 to unicellular cyanobacteria and isolation of receptor material from *Anacystis nidulans*. J Virol 25:164–174

Sandaa RA, Larsen A (2006) Seasonal variations in virus-host populations in Norwegian coastal waters: focusing on the cyanophage

community infecting marine *Synechococcus* spp. Appl Environ Microbiol 72:4610–4618

Sarma TA, Kaur B (1997) Characterization of host-range mutants of cyanophage N-1. Acta Virol 41:245–250

Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, Hess WR, Post AF, Hagemann M, Paulsen I, Partensky F (2009) Ecological genomics of marine picocyanobacteria. Microbiol Mol Biol Rev 73:249–299

Sedmak B, Carmeli S, Elersek T (2008) "Non-Toxic" cyclic peptides induce lysis of cyanobacteria – An effective cell population density control mechanism in cyanobacterial blooms. Microb Ecol 56:201–209

Sedmak B, Carmeli S, Pompe-Novak M, Tusek-Znidaric M, Grach-Pogrebinsky O, Elersek T, Zuzek MC, Bubik A, Frangez R (2009) Cyanobacterial cytoskeleton immunostaining: the detection of cyanobacterial cell lysis induced by planktopeptin BL1125. J Plankton Res 31:1321–1330

Shan J, Jia Y, Clokie MRJ, Mann NH (2008) Infection by the 'photosynthetic' phage S-PM2 induces increased synthesis of phycoerythrin in Synechococcus sp. WH7802. FEMS Microbiol Lett 283:154–161

Shane MS (1971) Distribution of blue-green algal viruses in various types of natural waters. Water Res 5:711–716

Shane MS, Cannon RE, DeMichele E (1972) Pollution effects on phycovirus and host algae ecology. J Water Pollut Control Fed 44:2294–2302

Sharon I, Alperovitch A, Rohwer F, Haynes M, Glaser F, Atamna-Ismaeel N, Pinter RY, Partensky F, Koonin EV, Wolf YI, Nelson N, Beja O (2009) Photosystem I gene cassettes are present in marine virus genomes. Nature 461:258–262

Sherman LA, Brown RM Jr (1978) Cyanophages and viruses of eukaryotic algae. In: Fraenkel-Conrat H, Wagner RR (eds) Comprehensive virology, vol 12. Plenum Press, New York, pp 145–234

Sigee DC, Glenn R, Andrews MJ, Bellinger EG, Butler RD, Epton HAS, Hendry RD (1999) Biological control of cyanobacteria: principles and possibilities. Hydrobiologia 395:161–172

Singh PK (1973) Occurrence and distribution of cyanophages in ponds, sewage and ricefields. Arch Microbiol 89:169–172

Sode K, Oozeki M, Asakawa K, Burgess JG, Matsunaga T (1994) Isolation of a marine cyanophage infecting the marine unicellular cyanobacterium, *Synechococcus* sp. NKBG 0429002. J Mar Biotechnol 1:189–192

Sode K, Oonari R, Oozeki M (1997) Induction of a temperate marine cyanophage by heavy metal. J Mar Biotechnol 5:178–180

Stewart FM, Levin BR (1984) The population biology of bacterial viruses: why be temperate? Theor Popul Biol 26:93–117

Stoddard LI, Martiny JBH, Marston MF (2007) Selection and characterization of cyanophage resistance in marine *Synechococcus* strains. Appl Environ Microbiol 73:5516–5522

Strom SL (1991) Growth and grazing rates of the herbiverous dinoflagellate *Gymnodinium* sp. from the open sub-arctic Pacific Ocean. Mar Ecol Prog Ser 78:103–112

Stuart RK, Dupont CL, Johnson DA, Paulsen IT, Palenik B (2009) Coastal strains of marine *Synechococcus* species exhibit increased tolerance to copper shock and a distinctive transcriptional response relative to those of open-ocean strains. Appl Environ Microbiol 75:5047–5057

Sullivan MB, Waterbury JB, Chisholm SW (2003) Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. Nature 424:1047–1051

Sullivan MB, Coleman M, Weigele P, Rohwer F, Chisholm SW (2005) Three *Prochlorococcus* cyanophage genomes: signature features and ecological interpretations. PLoS Biol 3:e144

Sullivan MB, Lindell D, Lee JA, Thompson LR, Bielawski JP, Chisholm SW (2006) Prevalence and evolution of core photosystem II genes in marine cyanobacterial viruses and their hosts. PLoS Biol 4:e234

Sullivan MB, Coleman ML, Quinlivan V, Rosenkrantz JE, DeFrancesco AS, Tan G, Fu R, Lee JA, Waterbury JB, Bielawski JP, Chisholm SW (2008) Portal protein diversity and phage ecology. Environ Microbiol 10:2810–2822

Sullivan MB, Krastins B, Hughes JL, Kelly L, Chase M, Sarracino D, Chisholm SW (2009) The genome and structural proteome of an ocean siphovirus: a new window into the cyanobacterial 'mobilome'. Environ Microbiol 11:2935–2951

Sullivan MB, Huang KH, Ignacio-Espinoza JC, Berlin AM, Kelly L, Weigele PR, DeFrancesco AS, Kern SE, Thompson LR, Young S, Yandava C, Fu R, Krastins B, Chase M, Sarracino D, Osburne MS, Henn MR, Chisholm SW (2010) Genomic analysis of oceanic cyanobacterial myoviruses compared with T4-like myoviruses from diverse hosts and environments. Environ Microbiol 12: 3035–3056

Suttle CA (2000) Cyanophages and their role in the ecology of cyanobacteria. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 563–589, 669 pp

Suttle CA, Chan AM (1993) Marine cyanophages infecting oceanic and coastal strains of *Synechococcus* – abundance, morphology, cross-infectivity and growth-characteristics. Mar Ecol Prog Ser 92:99–109

Suttle CA, Chan AM (1994) Dynamics and distribution of cyanophages and their effect on marine Synechococcus spp. Appl Environ Microbiol 60:3167–3174

Tai V, Palenik B (2009) Temporal variation of *Synechococcus* clades at a coastal Pacific Ocean monitoring site. ISME J 3:903–915

Tarao M, Jezbera J, Hahn MW (2009) Involvement of cell surface structures in size-independent grazing resistance of freshwater Actinobacteria. Appl Environ Microbiol 75:4720–4726

Thingstad TF (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnol Oceanogr 45:1320–1328

Thompson LR, Zeng Q, Kelly L, Huang KH, Singer AU, Stubbe JA, Chisholm SW (2011) Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. Proc Natl Acad Sci USA 108(39):E757–E764

Tijdens M, Hoogveld HL, Kamst-van Agterveld MP, Simis SGH, Baudoux AC, Laanbroek HJ, Gons HJ (2008a) Population dynamics and diversity of viruses, bacteria and phytoplankton in a shallow eutrophic lake. Microb Ecol 56:29–42

Tijdens M, van de Waal DB, Slovackova H, Hoogveld HL, Gons HJ (2008b) Estimates of bacterial and phytoplankton mortality caused by viral lysis and microzooplankton grazing in a shallow eutrophic lake. Freshw Biol 53:1126–1141

Tucker S, Pollard P (2005) Identification of cyanophage Ma-LBP and infection of the cyanobacterium *Microcystis aeruginosa* from an Australian subtropical lake by the virus. Appl Environ Microbiol 71:629–635

van der Oost J, Jore MM, Westra ER, Lundgren M, Brouns SJJ (2009) CRISPR-based adaptive and heritable immunity in prokaryotes. Trends Biochem Sci 34:401–407

van Hannen EJ, Zwart G, van Agterveld MP, Gons HJ, Ebert J, Laanbroek HJ (1999) Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. Appl Environ Microbiol 65:795–801

Van Wichelen J, van Gremberghe I, Vanormelingen P, Debeer AE, Leporcq B, Menzel D, Codd GA, Descy JP, Vyverman W (2010) Strong effects of amoebae grazing on the biomass and genetic structure of a *Microcystis* bloom (Cyanobacteria). Environ Microbiol 12(10):2797–2813

Waldor MK, Mekalanos JJ (1996) Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 272:1910–1914

Wang K, Chen F (2008) Prevalence of highly host-specific cyanophages in the estuarine environment. Environ Microbiol 10:300–312

Wang JD, Sanders GM, Grossman AD (2007) Nutritional control of elongation of DNA replication by (p)ppGpp. Cell 128:865–875

Wang GH, Murase J, Asakawa S, Kimura M (2009a) Unique viral capsid assembly protein gene (*g20*) of cyanophages in the floodwater of a Japanese paddy field. Biol Fertil Soils 46:93–102

Wang GH, Murase J, Asakawa S, Kimura M (2009b) Novel cyanophage photosynthetic gene psbA in the floodwater of a Japanese rice field. FEMS Microbiol Ecol 70:79–86

Wang GH, Asakawa S, Kimura M (2011) Spatial and temporal changes of cyanophage communities in paddy field soils as revealed by the capsid assembly protein gene g20. FEMS Microbiol Ecol 76: 352–359

Waterbury JB, Valois FW (1993) Resistance to co-occurring phages enables marine *Synechococcus* communities to coexist with cyanophages abundant in seawater. Appl Environ Microbiol 59: 3393–3399

Waterbury JB, Watson SW, Guillard RRL, Brand LE (1979) Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. Nature 277:293–294

Weigele PR, Pope WH, Pedulla ML, Houtz JM, Smith AL, Conway JF, King J, Hatfull GF, Lawrence JG, Hendrix RW (2007) Genomic and structural analysis of Syn9, a cyanophage infecting marine *Prochlorococcus* and *Synechococcus*. Environ Microbiol 9: 1675–1695

Wiggins BA, Alexander M (1985) Minimum bacterial density for bacteriophage replication: implications for significance of bacteriophages in natural ecosystems. Appl Environ Microbiol 49:19–23

Wildschutte H, Wolfe DM, Tamewitz A, Lawrence JG (2004) Protozoan predation, diversifying selection, and the evolution of antigenic diversity in Salmonella. Proc Natl Acad Sci USA 101: 10644–10649

Wilhelm SW, Matteson AR (2008) Freshwater and marine virioplankton: a brief overview of commonalities and differences. Freshw Biol 53:1076–1089

Wilhelm SW, Carberry MJ, Eldridge ML, Poorvin L, Saxton MA, Doblin MA (2006) Marine and freshwater cyanophages in a Laurentian Great Lake: evidence from infectivity assays and molecular analyses of g20 genes. Appl Environ Microbiol 72:4957–4963

Williamson SJ, Paul JH (2004) Nutrient stimulation of lytic phage production in bacterial populations of the Gulf of Mexico. Aquat Microb Ecol 36:9–17

Williamson SJ, Rusch DB, Yooseph S, Halpern AL, Heidelberg KB, Glass JI, Andrews-Pfannkoch C, Fadrosh D, Miller CS, Sutton G, Frazier M, Venter JC (2008) The Sorcerer II global ocean sampling expedition: metagenomic characterization of viruses within aquatic microbial samples. PLoS One 3:e1456

Wilson WH, Joint IR, Carr NG, Mann NH (1993) Isolation and molecular characterization of 5 marine cyanophages propagated on *Synechococcus* sp. strain WH7803. Appl Environ Microbiol 59:3736–3743

Wilson WH, Carr NG, Mann NH (1996) The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. WH7803. J Phycol 32:506–516

Winter C, Bouvier T, Weinbauer MG, Thingstad TF (2010) Trade-offs between competition and defense specialists among unicellular planktonic organisms: the "killing the winner" hypothesis revisited. Microbiol Mol Biol Rev 74:42–57

Xu X, Khudyakov I, Wolk CP (1997) Lipopolysaccharide dependence of cyanophage sensitivity and aerobic nitrogen fixation in *Anabaena* sp. strain PCC 7120. J Bacteriol 179:2884–2891

Yoshida M, Yoshida T, Kashima A, Takashima Y, Hosoda N, Nagasaki K, Hiroishi S (2008a) Ecological dynamics of the toxic bloom-forming cyanobacterium *Microcystis aeruginosa* and its cyanophages in freshwater. Appl Environ Microbiol 74:3269–3273

Yoshida T, Nagasaki K, Takashima Y, Shirai Y, Tomaru Y, Takao Y, Sakamoto S, Hiroishi S, Ogata H (2008b) Ma-LMM01 infecting toxic *Microcystis aeruginosa* illuminates diverse cyanophage genome strategies. J Bacteriol 190:1762–1772

Yoshida M, Yoshida T, Yoshida-Takashima Y, Kashima A, Hiroishi S (2010) Real-time PCR detection of host-mediated cyanophage gene ranscripts during infection of a natural *Microcystis aeruginosa* population. Microbe Environ 25:211–215

Zeidner G, Bielawski JP, Shmoish M, Scanlan DJ, Sabehi G, Beja O (2005) Potential photosynthesis gene recombination between *Prochlorococcus* and *Synechococcus* via viral intermediates. Environ Microbiol 7:1505–1513

Zeng QL, Chisholm SW (2012) Marine viruses exploit their host's two-component regulatory system in response to resource limitation. Curr Biol 22(2):124–128

Zhao FQ, Zhang XW, Liang CW, Wu JY, Bao QY, Qin S (2006) Genome-wide analysis of restriction-modification system in unicellular and filamentous cyanobacteria. Physiol Genomics 24:181–190

Zhong Y, Chen F, Wilhelm SW, Poorvin L, Hodson RE (2002) Phylogenetic diversity of marine cyanophage isolates and natural virus communities as revealed by sequences of viral capsid assembly protein gene g20. Appl Environ Microbiol 68:1576–1584

Zwart G, Kamst-van Agterveld MP, van der Werff-Staverman I, Hagen F, Hoogveld HL, Gons HJ (2005) Molecular characterization of cyanobacterial diversity in a shallow eutrophic lake. Environ Microbiol 7:365–377

Zwirglmaier K, Jardillier L, Ostrowski M, Mazard S, Garczarek L, Vaulot D, Not F, Massana R, Ulloa O, Scanlan DJ (2008) Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ Microbiol 10:147–161

Zwirglmaier K, Spence E, Zubkov MV, Scanlan DJ, Mann NH (2009) Differential grazing of two heterotrophic nanoflagellates on marine *Synechococcus* strains. Environ Microbiol 11:1767–1776

Part IV

The Organisms

Rivulariaceae

22

Brian A. Whitton and Pilar Mateo

Contents

B.A. Whitton (✉)
School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK
e-mail: b.a.whitton@durham.ac.uk

P. Mateo
Departamento de Biologia, Facultad de Ciencias, Universidad Autonoma de Madrid, 28049 Madrid, Spain
e-mail: pilar.mateo@uam.es

Summary

The Rivulariaceae are treated here as all the cyanobacteria which have trichomes with a marked taper and a basal heterocyst for much of their growth cycle. Molecular sequencing of *Calothrix* and *Rivula*ria shows that these are heterogeneous and this probably also applies to *Dichothrix* and *Gloeotrichia.* The unispecific *Isactis* has received little study, but is morphologically close to some *Calothrix*. These should all be treated as form-genera for ecological descriptive purposes until more sequencing studies have been made. *Sacconema* and *Gardnerula* are not considered distinct enough to be treated as distinct genera. Colony formation occurs by aggregation of hormogonia and this may lead to the inclusion of more than one genotype.

The group as a whole occurs in environments with highly variable P concentrations, usually short periods of relatively high ambient P followed by much longer periods of low P; a few possible exceptions are discussed. Typically organic P exceeds inorganic P and the Rivulariaceae as a whole are especially efficient at using organic phosphates. N_2 fixation in Rivulariaceae is highest during the period of high P. In the case of *Gloeotrichia echinulata*, which sometimes forms blooms in lakes, P acquisition takes place mainly while the organism is growing near the sediments. Its competitive success is favoured by a combination of P-rich sediments and relatively low P concentrations in the water.

Long colourless multicellular hairs are formed by many species, including all those with distinct hemispherical and spherical colonies. The hairs not only enhance the surface area for phosphatase activities, but also aid P acquisition from environments where the ambient P concentration is

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mostly low, but with occasional much higher pulses. Although most phosphatase activities take place at the cell surface, some activity often occurs in sheaths and probably also inside the cytoplasmic membrane of older hair cells, suggesting that organic phosphates can sometimes pass through the membrane.

Sufficient is known about the relationship between morphology and the environment in the Rivulariaceae to make them excellent environmental indicators. Many of the morphological characters used for classical taxonomic descriptions are expressed only when the organisms are P-limited, but some culture collections and many experimental studies have used media with very high P concentrations, making past interpretation of results doubtful, if not wrong.

22.1 Introduction

A number of general accounts of blue-green algae in the nineteenth century grouped genera with obviously tapered trichomes into the family Rivulariaceae, with *Rivularia haematites* the type species. Geitler (1932) summarized the earlier information, while Desikachary (1959) provided a succinct definition; "Trichomes with a single row of cells, apices generally attenuated or tapering in hair, unbranched or false-branched, sometimes with a distinct intercalary meristematic zone and trichothallic growth; hair with elongated more or less vacuolated cells; heterocysts present or absent, when present basal, intercalary heterocysts also present in some; hormogonia present, akinetes present or absent, when present single or in series". The 12 genera listed by Geitler and Desikachary included several without heterocysts such as *Homoeothrix*, but, even before molecular data started to make it possible to assess relationships more critically, most researchers assumed that non-heterocystous species were different.

The heterocystous Rivulariaceae include organisms found widely in nature and are in some cases visually conspicuous. Perhaps the best known is the planktonic *Gloeotrichia echinulata* (Sect. 22.5.4; Fig. 22.1), which can form blooms, but other species of *Gloeotrichia, Dichothrix* and *Rivularia* form conspicuous attached or floating colonies in shallow waters (Sect. 22.5.3). This chapter tries to establish to what extent the heterocystous Rivulariaceae are a uniform group phylogenetically and ecologically. At the same time it sets out to explain how phenotypic differences influence the success of a particular genus or species, and conversely, the extent to which one can interpret the environmental features of a site from the morphology of its Rivulariaceae. The reader needs to know some of the older literature in order to understand the argument.

Fig. 22.1 Young planktonic colonies of *Gloeotrichia echinulata* (Photo P.V. York, with permission)

22.2 Morphology

22.2.1 Morphology and Classical Taxonomy

The most widely accepted heterocystous genera of Rivulariaceae in classical floras (i.e. those based on International Code of Botanical Nomenclature) have trichomes with a basal heterocyst, a distinct taper to the trichome and a sheath enclosing everything but the heterocyst. *Calothrix* includes all the forms growing as individual filaments or ill-defined colonies. Four genera form distinct colonies, with *Gloeotrichia* separated by its ability to form akinetes. The others are distinguished by colony morphology, which is determined by the pattern formed by hormogonia when they first aggregate and also by the division pattern during colony growth. *Rivularia* colonies have more or less parallel sheathed trichomes inside hemispherical, subspherical or spherical colonies; the trichomes often have false branches. *Isactis* forms spreading colonies in the marine intertidal, with sparsely branched trichomes perpendicular to underlying rock surface. *Dichothrix* forms small colonies of various shapes, mostly cushions or dense tufts; trichome branching is conspicuous and there are often many trichomes inside one sheath. Many, but not all, freshwater *Dichothrix* and *Rivularia* become partially calcified (Fig. 22.2).

The distinctions between genera are not always clear-cut, such as between *Dichothrix* and *Rivularia*, but the names still prove useful in floristic accounts of natural communities. However, Rippka et al. (1979) included all heterocystous cyanobacteria with a low or high degree of tapering in *Calothrix* on the grounds that colony appearance in feral samples seemed to be a doubtful taxonomic trait. Only a brief justification for this statement was given and in any

Fig. 22.2 (**a, b**) ***Dichothrix baueriana*** in River Caher, draining The Burren, Co. Clare, Ireland, June 2006: (**a**) Upstream view of calcified boulders; (**b**) close-up of the colonies during a period of low flow; showing growth on the boulder in the zone influenced by recent fluctuations in water level; (**c**) ***Rivularia*** colonies among developing calcareous crust on boulder in Loch Nam Bakgan, South Uist, Outer Hebrides (Scotland), September 2009; colonies up to 8 mm diameter, with obvious calcification inside them (Photos B.A.W.)

case it is doubtful how many of the cultures had been observed closely when the organism was first sampled from nature. Concern about the reliability of names in culture collections using P-rich medium (Chap. 1) is especially relevant to the Rivulariaceae due to the need for the organisms to be at least moderately P-limited to express their characteristic morphological features (Whitton 1987, 1989, 2008, 2009; Berrendero et al. 2008; Sect. 22.4). Such concern extends to the names associated with sequence data deposited in GenBank.

22.2.2 Range of Form

22.2.2.1 Tapering and Hairs

The tapered trichome has a terminal heterocyst at the wider end and growth is often localized in a region near, but not immediately adjacent, to the heterocyst. Although the term 'meristematic' is often used in descriptions of Rivulariaceae to indicate this region, its position and the extent to which it is localized varies during growth. It is suggested that the region corresponds to where longitudinal gradients of N and P in the trichome (see below) interact at any one time to provide optimum growth conditions. The region is most distinct in species where the trichome becomes much wider close to the heterocyst, giving the appearance of a spindle-shaped swelling (Schwenender 1894; Friedmann 1956).

Many species can form multicellular hairs, structures which were defined by Sinclair and Whitton (1977a) as "a region of the trichome where the cells are much narrower, elongated, highly vacuolated and usually apparently colourless". This definition fits the way the term is used for descriptive purposes in most floras, including all but one of the species in Geitler (1932). However, other species occur with long tapered structures which resemble hairs in outline, but the cells retain their chlorophyll and do not elongate; these are quite common, at least in the subtropics. An example, *Calothrix* D764, is discussed in Sect. 25.2.2. The only exception in Geitler (1932), *C. kossinskajae*, is somewhat like this, but the figures show a scarcely tapered trichome which contracts over one or two cells to a very long extension of the main trichome, but only about one-third of its width. If (as seems likely) this gives rise to hormogonia, they would be little more than 1 μm wide.

All the descriptions of species in the genera forming distinct colonies show hairs. In the case of *Calothrix*, 56 out of the 78 species (72%) reported in the taxonomic literature surveyed by Kirkby and Whitton (1976) formed hairs, while

82 of 103 (81%, omitting a few uncertain species) did so in the names in CyanoDB.cz (July 2011). However, only 7 out of 29 *Calothrix* strains (24%) in culture did so, in spite of being tested under a range of environmental conditions known to lead to hair formation (Sinclair and Whitton 1977a). This raises the possibility that isolation procedures may be selecting against hair-forming strains. The hair cells of *Gloeotrichia echinulata* mostly retain their gas vacuoles (Smith and Peat 1967).

In the study by Sinclair and Whitton (1977a), P limitation enhanced tapering in all 34 Rivulariaceae strains tested, but only 12 strains developed hairs. Even in the presence of combined N, P-limitation still led to the formation of hairs in these strains (Sinclair and Whitton 1977b). Three strains formed some hairs under P-rich conditions, although hair frequency increased with increasing P limitation (Sinclair and Whitton 1977a). Much shorter hairs were formed under Fe deficiency in seven strains and under Mg deficiency in one strain, but there was no response to Ca, Mo or SO_4 deficiencies. Strains lacking the ability to form hairs had all been described as *Calothrix* in the culture collections from which they came. In addition to the uncertainty as to whether or not they possessed hairs when originally collected, there is also the possibility that they might have lost this ability during repeat subculture in a P-rich medium. However, when *Gloeotrichia* aff. *pisum* colonies with trichomes showing obvious hairs were removed from deepwater rice plants in Bangladesh, all attempts to isolate trichomes able to form hairs in culture failed (Aziz 1985). In addition to nutrient limitation, methods tested without success included changing the light and temperature regimes, spectral composition of the light and incubation in various organic phosphates as the sole P source. It remains uncertain whether an important factor was overlooked or whether the trichomes isolated were not representive of the main colony. Similarly, during isolation of three *Calothrix* species from an Iraqi marsh rice field, *C. fusca* and *C. parietina* both formed hairs in enrichment culture, but, once they were brought into axenic culture, *C. parietina* failed to do so (Al-Mousawi and Whitton 1983).

Cultures grown under increasingly P-limited conditions cease to produce hormogonia. The apical cells then start to develop intrathylakoidal vacuoles, which remain small in species which do not form hairs, but continue to increase in size and lose their photosynthetic pigments in species forming hairs. Formation (where present) can continue for a long period. The hair cells consist largely of vacuoles originating from intrathylakoidal vacuoles and in very long hairs each cell is largely filled by a single vacuole. *Rivularia* and other colonial forms with hairs which persist for many months usually have a long transition zone from typical vegetative cell to cells with increasingly larger vacuoles and then to the colourless hair cells (Wood 1984; Wood et al. 1986).

Multicellular hairs which appear morphologically similar also occur in at least some species of most genera of Stigonematales, the similarity being especially marked in *Nostochopsis* and *Brachytrichia*. The ends of the trichomes of *Aphanizomenon flos-aquae* often taper into short hair-like lengths, while several species of the non-heterocystous *Homoeothrix* and *Ammatoidea* also form hairs. This indicates that multicellular hairs have evolved many times in cyanobacteria, just as they have in eukaryotic algae (Whitton 1988).

22.2.2.2 Heterocyst

Although all Rivulariaceae can form terminal heterocysts, some also form intercalary ones. These have been observed in many species, but occur much more frequently in some than others. Polarity of the terminal cell is established before the hormogonium is released from the mother trichome. The heterocyst always develops in the cell adjacent to the main trichome i.e. basal, even though the cell furthest from it might be expected to be the most N-limited (Whitton 1987). In contrast to the terminal heterocyst, an intercalary heterocyst has polar nodules (cyanophycin) on both sides, so presumably fixed N can pass in both directions and thus permit further growth in both parts of the trichome.

Particularly in *Rivularia*, a new heterocyst is sometimes developed above the current one and the old one starts to collapse, eventually showing with the light microscope little more than the wall. Typically the new heterocyst has only a single polar nodule and thus becomes the terminal heterocyst. Growth of *Calothrix parietina* D184 (= PCC 7713) in medium without Fe led to repeat formation of new single-pored heterocysts, with rapid degeneration of the old one (Douglas et al. 1986). The new heterocyst was often separated from the old one by necridial cells. Several illustrations in the literature suggest that the new heterocyst may in some cases be intercalary, though this has never been observed by the authors; if it does occur, this might permit higher rates of N_2 fixation. Large *Rivularia* colonies, which have already grown for several years, sometimes have as many as eight remnant heterocysts beneath the normal terminal one. Presumably each new heterocyst forms as a response to a change in environmental conditions. One such possible response is a loss of the ability of the heterocyst to fix N_2 under prolonged periods of high ambient combined N, resulting in the need for a new one to be formed the next time N_2 fixation becomes important. All but one of 34 Rivulariaceae (representing all four genera) lost all heterocysts when cultured with 10 mM $NaNO_3$ (Sinclair and Whitton 1977b). However, this concentration of combined N is much higher than likely to occur in most environments. No studies have yet been made on the effects of fluctuating concentrations of combined N supplied at more realistic concentrations.

Another possible response is the formation of a new heterocyst due to the deficiency of an element important in

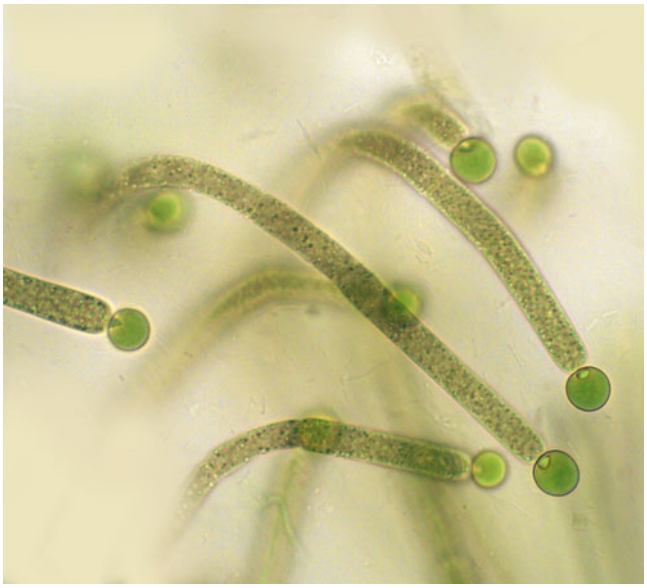

Fig. 22.3 Trichomes near the centre of a *Gloeotrichia pisum* colony. The bright coloured heterocysts often occur in members of the Rivulariaceae attached to submerged plants, sometimes green or blue-green (as shown here), but other times bright blue (Photo C. F. Carter, with permission)

heterocyst functioning. The new terminal heterocyst is often smaller than the original one. Tests on the influence of Mg, Ca, Fe, Mo or S deficiencies in 13 *Calothrix* strains showed a particularly marked effect of Mo deficiency on heterocyst frequency, the increase being at least four-fold in each case (Sinclair and Whitton 1977a). Ca and Fe deficiencies also led to obvious increases in heterocyst frequency in most strains. The extra heterocysts developed in both basal and intercalary positions, but most of the latter formed only a single polar nodule, thus leading to a 'terminal' heterocyst in the middle of the trichome. The hypothesis is suggested that in these cases the replacement of one type of heterocyst by another may be due to a shift in the type of electron transport system. Presumably some of the element in short supply is transported to the rest of the trichome, thus aiding the formation of heterocysts elsewhere.

It is over a century since Fritsch (1907a, b) first commented on the tendency for heterocysts in tropical Rivulariaceae to be blue and subsequent studies have shown blue heterocysts to be widespread, especially in ponds and rice fields (Whitton 1987). This also occurs in temperate regions, although less widely. In other cases they may be bright green (Fig. 22.3) rather than blue. Studies on *Calothrix* D603 isolated from the surface of a deepwater rice plant in Bangladesh showed that the blue colour increased as cultures became older and trichomes increased in length (Whitton et al. 1986). This is the exact opposite of the situation with some *Anabaena*, especially tropical strains, where the blue colour persists for some time after differentiation, but eventually disappears. Addition of NH_4-N to young *Calothrix* D603 cultures grown in the absence of combined N led to about 20% heterocysts turning an obvious blue. In shaded forest habitats *Calothrix* and *Dichothrix* with pink trichomes have bright blue heterocysts, indicating the absence of phycoerythrin in these heterocysts (B.A.W., unpublished). However, the heterocysts of a lichenized *Calothrix* form phycoerythrin (see Sect. 22.6.2)

The heterocysts of several Rivulariaceae have been reported to germinate and produce short germlings. This was described in most detail for *Gloeotrichia raciborskii* and *Rivularia manginii* by Desikachary (1946) and a non-sporulating mutant of *Gloeotrichia ghosei* by Singh and Tiwari (1970). In all cases the cyanophycin granule by the cross-wall disappeared at an early stage. Even where there is not such a marked reversal of the initial differentiation to form a heterocyst, heterocysts of Rivulariaceae sometimes reform internal structures which have been lost in their initial differentiation (Whitton 1987), such as carboxysomes in *Calothrix* D603 (Whitton et al. 1986). It was suggested that the heterocyst may have switched back from N_2 fixation to CO_2 fixation. Another possible explanation for blue heterocysts is that the phycocyanin is behaving as a N store; perhaps it can be mobilized more rapidly than cyanophycin. Either or both features would be useful for an organism living in a highly variable environment.

22.2.2.3 Hormogonia

Hormogonia typically develop at the end of the trichome or, in species with a hair, immediately below this; in the latter case the hair becomes detached and disintegrates. The main zone of cell division typically shifts from nearer the base of the trichome to nearer the apex. A necridial cell is formed at the base of each hormogonium in *Calothrix* D184 and D550 (Wood 1984; Wood et al. 1986) and this is apparently the general situation in Rivulariaceae. Perhaps formation of the necridial cell helps to determine that the adjacent cell will differentiate into a heterocyst.

The rice-field isolate *Gloeotrichia* D613 (see Sect. 22.4.2) showed a distinctive pattern when grown under a relatively high P concentration (Aziz and Whitton 1989). The trichome developed as cell groups, each of which had originated from one mother cell, something previously reported by Schwendener (1894). Typically the cell group of *Gloeotrichia* D613 consisted of 8 cells; if so, cell 8 distant from the apex differentiated into a necridium, releasing the other 7 cells as a cyanophycin-rich hormogonium. Subsequent to its release, the lowermost cell of the hormogonium differentiated into a heterocyst about 1 day later. Seven is the typical cell number in the hormogonia of many Rivulariaceae, although in some strains there is considerable variation (Fig. 22.4).

Hormogonia of Rivulariaceae usually contain all three types of storage body, polyglucoside, cyanophycin and polyphosphate. In *C. parietina* D184 cultured in N-free

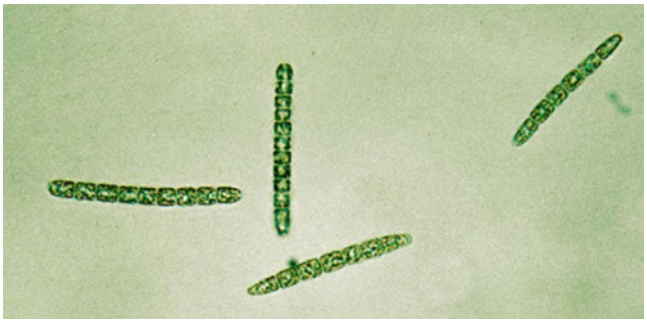

Fig. 22.4 Hormogonia from *Rivularia biasolettiana* (B.A.W.)

medium cyanophycin was the most rapid to decrease (Wood et al. 1986). Few, if any, divisions occur in a hormogonium between the time it is differentiated and the time it separates and often not until the first heterocyst is formed. However, subsequent to their release, *Gloeotrichia echinulata* hormogonia can elongate and then form necridia and fragment into short lengths (Maxwell 1974). Something similar was reported by Adamec et al. (2005) for *Calothrix elenkinii*, though their growth conditions make it hard to interpret the significance. When a culture grown in BG11 medium (high combined N and high P) was transferred to N-free medium and then exposed to green light, there was a transient development of a trichome cell with high fluorescence initiated by phycoerythrin absorption; this was followed by bleaching within about 20 min. These necridia permitted trichome fragmentation. The authors suggested that this allowed filaments to escape unfavourable environmental conditions; in this particular case, however, there was no nutrient shortage.

Hormogonia can glide on surfaces, including other hormogonia and cyanobacterial trichomes, an important feature in colony formation (see below). Hormogonia of *Calothrix* in streams, ponds and rice fields often have gas-vacuoles, even though these are absent in the mature population; it is unclear whether the presence of gas vacuoles influences gliding. Even without gas vacuoles, hormogonia of pond populations can persist in the plankton for some time and it needs careful observation to separate them from *Oscillatoria*. The transient nature of the gas vacuoles was confirmed in the study of two strains by Campbell et al. (1993): 8 h after release of hormogonia, gas vesicle genes were expressed and phycobiliprotein genes repressed; by 24 h the converse was true. In addition, hormogonia can lose their ability to form gas vacuoles during prolonged subculture in the laboratory, as was shown for two *Calothrix* strains isolated from rice fields in Nepal (Vaidya 1989).

The addition of phosphate to P-limited cultures led to hormogonia formation in all the *Calothrix* and *Rivularia* strains tested (Sinclair and Whitton 1977a; Whitton 1987); addition of the missing element to cultures limited by other elements also led to hormogonia formation, though the response usually took longer than with P limitation. However, growth of *Calothrix parietina* D184 to Fe-limitation, followed by the addition of Fe, led to the release of gas-vacuolate hormogonia, though often with hair fragments still attached (Douglas et al. 1986). The study by Campbell et al. (1993) on *Calothrix* PCC 7601 and 7504 showed that transfer to fresh medium and incubation under red light led to hormogonia formation, but not green light. Use of inhibitors demonstrated that the opposing effects arose through differential excitation of photosystems I and II. Although the authors showed the importance of electron transport in regulating cell differentiation, assessing the ecological relevance of their results is more difficult. There is no mention of phosphate, but it seems likely that the cultures had been subcultured routinely under P-rich conditions and never encountered the alternation of P-limited and P-rich conditions characteristic of their natural environment. (Based on its name in the UTEX Culture Collection and published accounts, PCC 7601 is probably not *Calothrix*, but *Microchaet*e, though it has also been called *Fremyella* and *Tolypothrix* elsewhere! PCC 7504 was reported to be quite similar.)

Calothrix strains in culture subjected to P limitation and then left for long periods eventually start to lyse, though often not for many months. However, in at least two strains studied in Durham, short lengths in otherwise partially lysed cultures differentiated into healthy hormogonia (B.A.W., unpublished). Assuming the same behaviour occurs in some strains in nature, this would provide a means for a dying population to colonize new sites.

22.2.2.4 Akinete

The akinete develops next to the terminal heterocyst in most cases, but intercalary akinetes are frequent in some species. The akinete may develop from a single cell (Desikachary 1959) or a number of cells with breakdown of the cross-walls (e.g. *Gloeotrichia ghosei*: Claassen 1973). Akinete formation is a characteristic feature of *Gloeotrichia*, but also occurs in several species of *Calothrix* and in *Dichothrix gelatinosa* (Böcher 1946). Two species of *Calothri*x have been reported to form akinetes. The original illustration of *C. stagnalis* by Gomont (1895) shows individual filaments epiphytic on *Cladophora*, so it is clearly not just a loosely organized *Gloeotrichia* colony. The description of *C. santapaui* (Gonsalves and Kamat 1960) is less convincing, because the filaments were in a mucilaginous mass with other algae. In addition, the spreading layers of the sheath are more like those of some *Dichothrix*. In G. *ghosei*, the only akinete-forming strain of the 34 Rivulariaceae studied by Sinclair and Whitton (1977a), increased akinete formation occurred under P limitation.

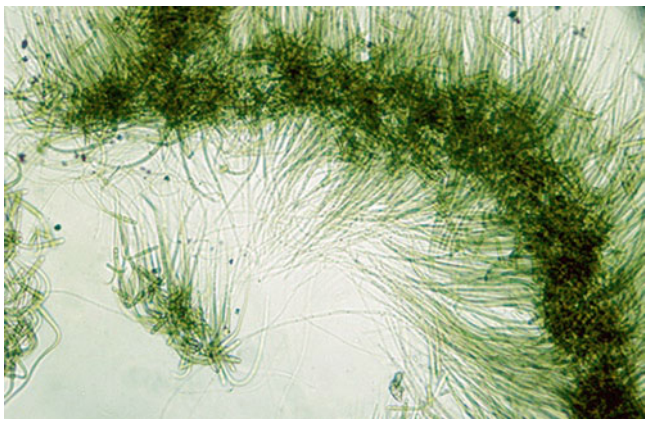

Fig. 22.5 Growth of *Calothrix parietina* D550 in culture, showing arrangement of trichomes following an initial period of hormogonial aggregation and subsequent commencement to form multicellular hairs when P-limited (Photo B.A.W.)

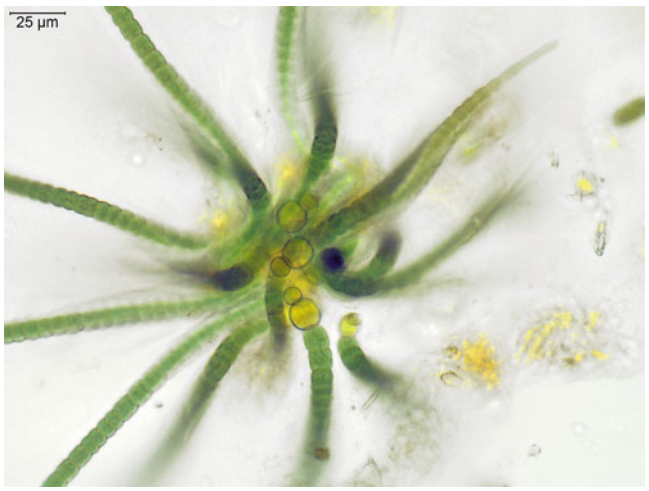

Fig. 22.6 Very early stage in the formation of an epiphytic *Gloeotrichia* colony (perhaps *G. pisum*). The orange-brown colour of the heterocysts probably indicates a high carotenoid content (Photo C. F. Carter, with permission)

22.2.2.5 Colony, Trichomes and Sheath

Rivularia, *Isactis*, *Gloeotrichia* and most species of *Dichothrix* all form colonies, while many species of *Calothrix* also form a macroscopic thallus with a characteristic appearance. For instance, the hormogonia of *C. pulvinata* attach themselves to parent filaments, leading to a tufted, bushy structure. In many other strains of *Calothrix* grown in liquid culture the released hormogonia aggregate to form tight clumps or, more usually, ropes (Fig. 22.5); observations on *C. parietina* D550 isolated from a stream showed that the most recently released hormogonia moved to the end of the rope (Livingstone and Whitton 1983). As growth proceeds, the aggregated hormogonia eventually separate slightly (B.A.W., unpublished). *Rivularia* sp. IAM-M-261 showed a positive phototropic response to white light (Katayama et al. 2007), but it is not known whether this is widespread in the Rivulariaceae.

De Bary (1863) was the first to report that aggregation of motile hormogonia into small non-motile groups was the initial sign of colony formation in *Rivularia*. Similar behaviour occurs in *Gloeotrichia echinulata* (Maxwell 1974) and *Gloeotrichia* aff. *pisum* (Aziz and Whitton, unpublished). After re-arrangement into a spherical shape, the trichomes start to differentiate (Fig. 22.6). Clonal cultures of colony-forming Rivulariaceae can sometimes form colonies, while others apparently do not (e.g. Chang 1979b), though in at least some cases this is merely due to excessively high phosphate concentration in the medium, as shown by Berrendero et al. (2008). However, other factors are probably involved in at least some cases. For instance, in a study of the behaviour of two marine Rivulariaceae strains transferred to laboratory culture, Darley (1967) found that aggregation of hormogonia only occurred if a large quantity was inoculated simultaneously.

Colony formation by a strain of *Rivularia biasolettiana* on agar involves an initial period of aggregation, followed by a period when no more trichomes are incorporated into the clump (B.A.W., unpublished). The trichomes then reorientate into a hemispherical or subspherical arrangement and heterocyst development in the basal cell of each trichome becomes visually obvious. If determination of the cell to become a heterocyst has already occurred before the hormogonium is released and occurs in the cell nearest the mother trichome, as observed for *Calothrix* D.256 (Sect. 22.2.2.2), rearrangement to form a colony would require this end of a moving trichome to be in front, the opposite of what happens when hormogonia are first released.

Initially each trichome develops a sheath, which is attached to the lowermost vegetative cell; it never surrounds the heterocyst. Differences in colony structure, which vary markedly between species of *Dichothrix*, result from differences in the way hormogonia become aggregated with the original after release from the mother trichome . There are also marked differences in sheath structure, which are especially obvious in *Dichothrix* (Fig. 22.7). The sheath stays close to the trichome in *D. orsiniana*, with only the colourless parts of the hair projecting from the end; its sheath varies considerably in the extent to which it is laminated. The sheaths of another widespread species, *D. gypsophila*, are not only strongly laminated, but the laminations splay open at the ends. Sheath thickness increased in all 34 Rivulariaceae strains when grown to P limitation and in 13 strains became dark brown due to scytonemin (Sinclair and Whitton 1977a). Other element deficiencies also led to thicker sheaths and in some cases scytonemin formation. Comparison of two *Calothrix* populations differing markedly in their scytonemin content

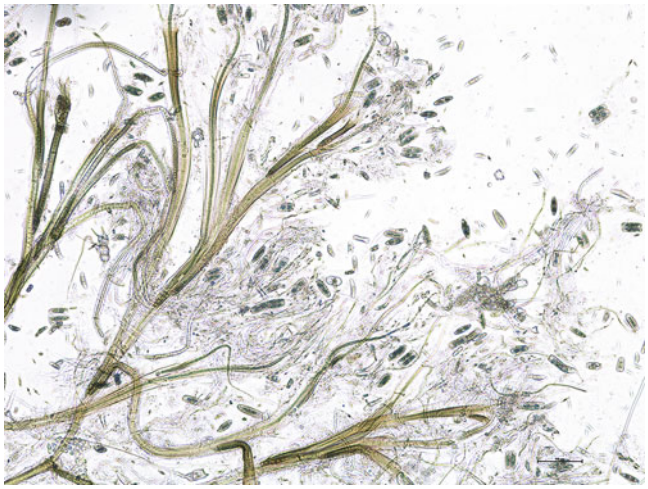

Fig. 22.7 *Dichothrix* (probably *D. gypsophila*) filaments in a mixed community developing into a stromatolitic crust on a boulder in Lough Corrib, Ireland; sample treated with dilute HCl. Other phototrophs include *Schizothrix fasciculata* and several pennate diatoms (Photo Bryan Kennedy, with permission). More views of this lake are shown in the attached online article by Kennedy, O'Grady and Whitton

from two Yellowstone thermal spring outflows indicated that the difference was partly genetic and partly environmental (Dillon and Castenholz 2003, Dillon et al. 2003). However, the sheath composition (with about 50% neutral sugars and also including 5% amino acids, small amounts of glucosamine and galacturonic acid) of *Calothrix parietina* D550 and *C. scopulorum* D256 in culture was not influenced by changes in the P or Fe content of the medium (Weckesser et al. 1988).

Three different morphological types of Rivulariaceae trichome were isolated from a single colony of *Rivularia biasolettiana* from a highly calcareous pond (Sinclair and Whitton 1977a). Molecular studies by Berrendero et al. (2008) also indicate that *Rivularia* colonies may include more than one genotype (see below).

22.3 Molecular Perspective on Taxonomy

22.3.1 Status of Classical Genera

A considerable number of molecular studies have been reported to help understanding of the phylogenetic relationships within the cyanobacteria in general or in particular groups. Most have been based on 16S rRNA gene sequences, with the interpretation on results relying on the fact that no horizontal transfer has so far been detected for this gene (Thomazeau et al. 2010). A variety of terms have been introduced to help interpretation of molecular results, such as form-genus in the 2001 edition of Bergey's Manual. As mentioned above, interpretation of such studies relies on the name given to a strain being that most suitable for the material when first removed from nature. It also relies on the trichome or clonal culture originating from that trichome being representative of the material isolated from nature. In the Rivulariaceae in particular there is a risk that either of these is wrong. Reliable identification of a P-rich culture based solely on morphological features is almost impossible and there is no way of knowing how representive a single trichome taken from a colony is of the whole colony.

Two genome sequences have been completed: *Calothrix desertica* PCC 7102 and *Calothrix* PCC 7103 (DOE Joint Genome Project website, May 2011). Both strains have been in culture collections for very long periods – over 60 years for *C. desertica*. Both belong to the groups able to form hairs (Sinclair and Whitton 1977a). *Calothrix* PCC 7103 was originally maintained as *Gloeotrichia* sp. and has also been called *Nodularia sphaerocarpa*, presumably when cultured in high nutrient medium. Neither appears to have been used for ecologically orientated studies.

Rippka et al. (2001a, b) considered the taxonomic diversity of *Calothrix* and *Rivularia*. Clusters within these genera were recognized by DNA-DNA hybridization studies using the methodology of Lachance (1981), supplemented in some cases by differences in pigment composition. Three clusters were distinguished within the 16 strains originally maintained as *Calothrix* (Rippka et al. 2001a). Cluster 1 (eight strains) showed a relatively high degree of genetic relatedness, although it could be divided into five subclusters. All but one strain (*C. marchica* PCC 7714 = D184) can form hairs. Cluster 2 (renamed from the cluster 3 of Rippka et al. 1979), which had only one strain, had been isolated from a sphagnum bog and was the only strain to form akinetes, when cultured in the absence of combined N. It conformed best, though not perfectly, to *C. stagnalis* Gomont. Subsequent phylogenetic analysis (Wilmotte and Herdman 2001) has shown that this strain (PCC 7507) fits in a different clade, where it shares a branch with *Cylindrospermum stagnale* 7417. The other cluster (seven strains), which were all marine isolates, was transferred to the form-genus *Rivularia* based on the fact that one of the strains had previously been identified by R. A. Lewin as *Rivularia* sp. Essentially this means that Rippka et al. (2001b) were treating freshwater tapered organisms with and without hairs as *Calothrix* and marine ones as *Rivularia*, irrespective of any colony structure. Because of uncertainty about strain names in the Pasteur Culture Collection, it is unclear whether any freshwater *Rivularia* (or *Dichothrix*) had been included in the study.

The study of Sihvonen et al. (2007), which was based on 16S rRNA sequencing, compared 42 strains of *Calothrix*, *Gloeotrichia* and *Tolypothrix*. This study had the advantage over Rippka et al. (2001a, b) in that 34 of the strains had been collected from brackish (mostly c. $5^0/_{00}$) and freshwater sites around the Baltic Sea and only eight were taken from the Pasteur Culture Collection. The three genera showed a

high level of genetic diversity and, in the case of organisms identified morphologically as *Calothrix*, the sequence differences were sufficiently large to suggest at least five different genera. There was no correlation between the phylogenetic clusters and site or habitat from which the strain had been isolated, nor did the nine strains able to produce hairs group together. Several strains clustered with 16S rRNA genes from other genera e.g. *Calothrix brevissima* IAM-M249 with *Tolypothrix* strains. The strongly tapering *Calothrix* strain BECID 18 did not cluster with any other strain and had the closest similarity (94%) with *Cyanospira rippkae* PCC 9501. A cluster comprising *Gloeotrichia echinulata* strains shared 98% sequence similarity, but were distant from the strains of the other genera. The authors also reported that a number of *Calothrix* strains from the Baltic Sea differentiated solitary akinetes or chains of akinetes. Although it seems clear that several organisms considered as *Calothrix* can form akinetes (Sect. 22.2), the comment by Sihvonen et al. suggests that their study may have included strains corresponding to *Gloeotrichia* based on classical taxonomic criteria.

Calothrix is also mentioned among various cyanobacteria in several 16S rRNA sequence studies dealing with a particular type of environment. Taton et al. (2006) isolated 59 cyanobacteria from 23 Antarctic lakes in order to characterize morphological and genotypic diversity (though sequence studies were only made on 56 strains). Based on morphology, 12 species were recognized, 4 of which are Antarctic endemics. Based on gene sequence, 21 OTUs (operational taxonomic units) were recognized, using the criterion of sequences having more than 97.5% similarity. These included 9 novel and 3 exclusively Antarctic OTUs. *Calothrix* was represented by one morphospecies and two molecular data analyses.

A project (Cuzman et al. 2010) on the biodiversity of phototrophic biofilms on fountains associated with monuments in Italy and Spain led to a comparison of 16S rRNA for 31 isolates with sequences of strains from other sources in the GenBank database. A neighbour-joining phylogenetic tree showed all seven *Calothrix* and *Rivularia* strains grouping more closely together than with any other genera. These included two *Calothrix* and one *Rivularia* from their own isolates.

Hongmei et al. (2005) reported a phylogenetic analysis of communities of mats of mostly filamentous cyanobacteria in the 42–53°C range from China, The Philippines and Thailand. Separation of 16S rRNA gene-defined genotypes from community DNA was achieved by DGGE, leading to the recovery of 36 sequences. Phylogenetic analyses indicated these formed novel thermophilic lineages distinct from their mesophilic counterparts in the case of seven genera, including the only *Calothrix* sampled. Srivastava et al. (2009) also used comparisons of DGGE bands from samples originating in (Uttar Pradesh) rice fields with GenBank database to characterize the cyanobacterial flora of low and high salinity sites. They included a list of the taxa observed by light microscopy and both this and the DGGE comparison indicated one *Rivularia* and one *Gloeotrichia*.

Phylogenetic analysis by Berrendero et al. (2008) used sequences from both 16S rRNA genes and an intergenic spacer (*cpcBA*-IGS) (see Sect. 22.3.2). A neighbour-joining tree based on 16S rRNA genes showed 35 Rivulariaceae grouped together, including all those isolated in their study. Data for two other strains listed as *Calothrix* in GenBank grouped with other genera. A further analysis based on 16S rRNA gene sequences (Berrendero et al. 2011) confirmed the diversity of *Calothri*x, but also their clear separation from eight *Tolypothrix* isolates and material of *T. penicillata*, all from rivers in Spain. The two groups could also be separated based on their morphology when cultured in a medium with 0.2 mg L^{-1} P, but not in standard BG-11_0 medium with 5 mg L^{-1} P.

Domínguez-Escobar et al. (2011) made a comparison based on not only almost the complete 16S rRNA gene, but also intergenic transcribed spacers and part of the 23S rRNA gene. Strains of *Calothrix*, *Rivularia* and *Tolypothrix* were isolated from diverse geographical regions and habitats. Based on results for their newly isolated strains and data for *Gloeotrichia* from Sihvonen et al. (2007), the authors made molecular clock estimates on the origin of the various genera. The methodology for doing this was based on a number of previous studies (e.g. Sanderson 2002) and involves approximations and estimates, such as the node defining cyanobacteria being fixed at ~2,700 Ma (million years ago) and a minimum age for the heterocystous cyanobacteria at ~1,618 Ma (Falcón et al. 2010). The latter value in particular differs considerably from the ~2,400 Ma for Nostocales suggested in Chap. 2 (Sect. 2.4.1.2), so the values of ~1,500 Ma for *Calothrix* and *Rivularia*, and 400–300 Ma for *Gloeotrichia* and *Tolypothrix* should be treated with caution. Nevertheless they provide a stimulus for further critical study.

22.3.2 Molecular Diversity Within Form-Genera

While studies described above include information on the form-genera *Calothrix* and *Rivularia*, several deal specifically with them. Shalini et al. (2008) using RAPD – PCR to assess the phylogenetic relatedness of 31 *Calothrix* strains from India and a reference strain (UTEX 379). A combination of 12 sets of primers generated 903 distinct polymorphic DNA fragments, indicating a wide range of variation among the strains. The highest correlation coefficient (0.821) was found for two strains which came from the same geographical location. The authors discussed the possible importance of geographical proximity in relatedness between strains, though it seems just as likely that environmental similarity is a key factor in this genus.

Berrendero et al. (2008) focussed on *Rivularia*, using colonies from five different rivers in Spain and one stream in the UK, all calcareous. They also used 13 strains isolated from calcareous rivers and one from a stream flowing over siliceous substrates. All were listed as 'strongly tapering (*Rivularia* or *Calothrix*)', but only *Calothrix* – no *Rivularia* – occurred in the siliceous stream. The methods included analysis of the phycocyanin operon (PC) and intervening intergenic spacer (*cpcBA*-IGS) and 16S rRNA gene sequences, together with molecular fingerprinting. The *cpcBA*-IGS analysis showed that all the sequences of environmental *Rivularia* colonies and rivulariacean-type isolates from calcareous rivers fell into one of three distinct genotype groups. Alignment of the sequences revealed 100% similarity of some isolated strains with the sequences in some field *Rivularia* colonies.

Genotype I was found in *Rivularia* colonies from the Endrinales and Matarraña rivers and strains isolated from these rivers, together with two other strains. Genotype II was found in isolates from the Endrinales, Muga and the siliceous stream. Genotype III was found in *Rivularia* colonies from the Alharabe, Muga and Red Sike (UK) and one isolate from another river. All three genotypes were reported for the Muga, though this may simply reflect the fact that, in addition to the colonies, six isolates came from this river. Culture of Genotype I isolates in a medium with relatively low phosphate concentration (0.2 mg L^{-1} P) led to *Rivularia*-like morphological characteristics, including secondary trichomes remaining in the mother sheath, lamellated sheath, confluent trichomes and a tendency to form spherical colonies. However, when Genotype II isolates were cultured in the same medium, they maintained *Calothrix*-like morphological characteristics and only formed irregular clumps or bundles of trichomes. These results suggest that Genotype I isolated strains belong to traditional *Rivularia* and Genotype II strains to traditional *Calothrix*.

Berrendero et al. cautioned that, because some cyanobacteria possess two or three PC operons (Golden 1995), their *cpcBA*-IGS analysis does not rule out the possibility of more than one PC being present, but only one sequenced. They therefore investigated this further using TGGE (temperature-gradient gel electrophoresis), which allows sequence-dependent separation of PCR products. The high sequence variability among the three genotypes, which gave rise to three clearly separated bands, allowed each genotype to be characterized by its corresponding fingerprint. There was only one band for each culture and this was consistent with the corresponding fingerprint. Colonies from the Alharabe and Red Sike, which best fitted *Rivularia biasolettiana*, corresponded to Genotype III. Colonies from the Blanco, Endrinales and Matarraña rivers all had bands corresponding to more than one genotype. Those from the Endrinales, which were typical *R. haematites*, had not only a band corresponding to Genotype I, but also one to Genotype II. The Blanco and Muga also had colonies with Genotype II, suggesting the presence of *Calothrix*-like filaments in the *Rivularia* colonies. Some colonies from the Blanco showed bands corresponding to both Genotypes I and III, suggesting that a single colony may include filaments of two different types ("species") of *Rivularia*.

The phylogenetic sequencing analysis, which formed part of the study by Berrendero et al., showed that the 31 strains in the Rivulariaceae group (see Sect. 22.3.1) fell into three distinct genotype clusters. The Genotypes I, II and III from studies on their own colonies and isolates each fitted into one of the three broader genotype clusters.

Apparently no similar studies have included *Dichothrix*, but the results would be of considerable interest, because some species are morphologically little more than complex forms of *Calothrix*, while others are hard to distinguish from *Rivularia*. A detailed analysis of *Gloeotrichia* would be even more interesting, because the planktonic *G. echinulata* differs considerably from other species in the genus, at least some of which seem little different from a *Rivularia* able to form akinetes (Sect. 22.5).

22.4 Physiology

22.4.1 Carbon and Nitrogen

In laboratory culture most Rivulariaceae appear to grow more slowly than filamentous species with untapered trichomes, though a *Gloeotrichia* aff. *pisum* culture from a Bangladesh deepwater rice field had a doubling time of 12.6 h under optimum growth conditions (Aziz 1985). There are a number of reports of photoheterotrophic growth and other physiological responses to sugars. When *Calothrix elenkinii* was grown on a light-dark cycle, glucose enhanced pigment production, especially β-carotene and chlorophyll (Prasanna et al. 2004a, b). Lebedeva et al. (2005) found that glucose enhanced phycocyanin production of *Calothrix* PCC 7601 under red light, but not phycoerythrin under green light. Dark heterotrophic growth with sucrose was reported for the two *Calothrix* strains included in the study by Khoja and Whitton (1971); glucose did not support heterotrophic growth of *Gloeotrichia echinulata* (Chang 1979a).

All (heterocystous) Rivulariaceae tested have proved capable of N_2 fixation and, in batch culture under continuous light, the highest rates have been reported several days after subculture to fresh medium. The rates are usually much higher in the light than the dark, whether measured in the laboratory (Stewart 1967; Chang and Blauw 1980; Al-Mousawi 1984) or the field. The rate dropped rapidly when intertidal *Rivularia atra* was transferred from light to dark (Khoja et al. 1984), a useful feature for this population which is sometimes smothered in sand for part of the tidal

cycle. This contrasts with the only slight initial change in rate shown by *Calothrix crustacea* mat on sand and *Rivularia* mat on silt at the edge of the lagoon of Aldabra Atoll (Potts and Whitton 1977). Diel studies by Hübel and Hübel (1974) of *C. scopulorum* and *Rivularia* on the Baltic Sea coast found that rates were usually 6–10 times higher at midday than in the night, though the difference was less marked during July and August, presumably due to the greater daytime accumulation of carbohydrate. The ratios between midday and midnight N_2 fixation in *R. biasolettiana* in Red Sike, Upper Teesdale, northern England, on two dates in August were similar (8–16% at night) (Livingstone et al. 1984). Overall about 400 mol CO_2 to 1 mol N_2 was fixed during daylight and the authors suggested that N_2 fixation may supply only a small percentage of its N requirement. However, another possibility is that N_2 fixation rates are higher in spring when ambient, mostly organic, P can be almost three orders of magnitude higher in this stream than in August (Livingstone and Whitton 1984). (Use of the term 'organic' for P fractions is explained by Whitton and Neal 2010). If so, then much of the C fixed in summer may be used in sheath formation. The upper intertidal organisms *Calothrix scopulorum* (Jones and Stewart 1969) and *Rivularia atra* (Reed and Stewart 1983) show greater inhibition under high salinity of nitrogenase than photosynthesis, and it was suggested that this may reflect channelling of fixed C into an osmoticum.

A comparison of nitrogenase activity of *Gloeotrichia pisum* colonies from the aquatic roots of deepwater rice plants in Bangladesh with a laboratory isolate (*Gloeotrichia* D613) showed a rapid fall in activity in both cases with the onset of dark conditions (Aziz and Whitton 1988). Nitrogenase activity was much higher when material grown in the dark for 12 h was transferred to the light than the highest rate found under continuous illumination. Longer periods of dark pretreatment led to only moderate increases in the period before peak nitrogenase activity was reached again. On transfer from light to dark, nitrogenase activity fell rapidly to very low values. Comparison in a Bangladesh deepwater rice field of the% total nitrogenase activity at night for *Gloeotrichia* cf *pisum* and *G. natans* (each on two different days) compared with that of four other cyanobacteria showed that *Gloeotrichia* always had much lower % values (Rother et al. 1988). Nitrogenase activity of *Calothrix* D764, another deepwater rice-field isolate, showed similar responses (Islam and Whitton 1992b). Rapid and marked responses to changes in light are important during the monsoon season and may be a feature of deepwater rice-field cyanobacteria, as many were found to show peak activity 6 h after dawn (Rother et al. 1988). This response of nitrogenase activity on transfer to light parallels the high rates of phosphate accumulation when P-deficient cyanobacteria are presented with phosphate (Healey 1982). The early literature on N_2 fixation rates by Rivulariaceae in the field, mainly marine sites, was summarized by Whitton (1987).

Marked changes in N composition were found during batch culture of *Calothrix* D764 (Islam and Whitton 1992b). The concentrations of chlorophyll, phycocyanin and phycoerythrin had all reached their highest values by the stage when the culture had reached 35% of its final yield. Soon afterwards there was a sharp decrease in nitrogenase activity, which subsequently persisted at the same rate for several weeks. 51% of the N present in pigments at the time they reached their peak values was transferred to other substances in old cultures, presumably cyanophycin and molecules associated with maximizing uptake of nutrients in limited supply.

There are a number of reports of biochemical features which appear to characterize the Rivulariaceae. For instance, in a study of heterocyst glycolipids, those of four *Calothrix* strains were dominated by a C_{28} rather than by the C_{26} carbon chain of the 17 Nostocaceae studied (Bauersachs et al. 2009). The functional and possible ecological significance of such molecular differences awaits study.

22.4.2 Phosphorus

The inhibitory effect of even moderate concentrations of inorganic phosphate noted by Fogg (1969) for *Gloeotrichia echinulata* seems to be widespread in Rivulariaceae; however, all the reported studies are for hair-forming species, so it is unclear whether this also applies to *Calothrix* strains incapable of forming hairs. The formation of hairs under P limitation (Sect. 22.2.2.3) was investigated further in *Calothrix parietina* D550 (Livingstone and Whitton 1983). Hairs are formed as the average value for cellular P falls to about 1% dry weight, when the filaments still appear healthy, indicating that the change occurs when the organism is only moderately P-limited. If phosphate is added to a culture with hairs, polyphosphate granules are formed rapidly in cells towards the base of the trichome and a few hours later several hormogonia start to differentiate at the apical end of the chlorophyll-containing part of the trichome. Light is not essential for polyphosphate granule formation in *Calothrix* D550, though it is for hormogonia formation.

Surface phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities of *C. parietina* D550 during batch culture commence at about the same stage of P limitation as hair formation (Livingstone and Whitton 1983); release of extracellular PMEase commences at the same time (Grainger et al. 1989), though in some other strains it commences later. No soluble extracellular PDEase is formed in *C. parietina* D550, nor in most other cyanobacteria and eukaryotic algae (Whitton et al. 2005).

Several staining techniques have been used to compare differences in distribution of PMEase activity between

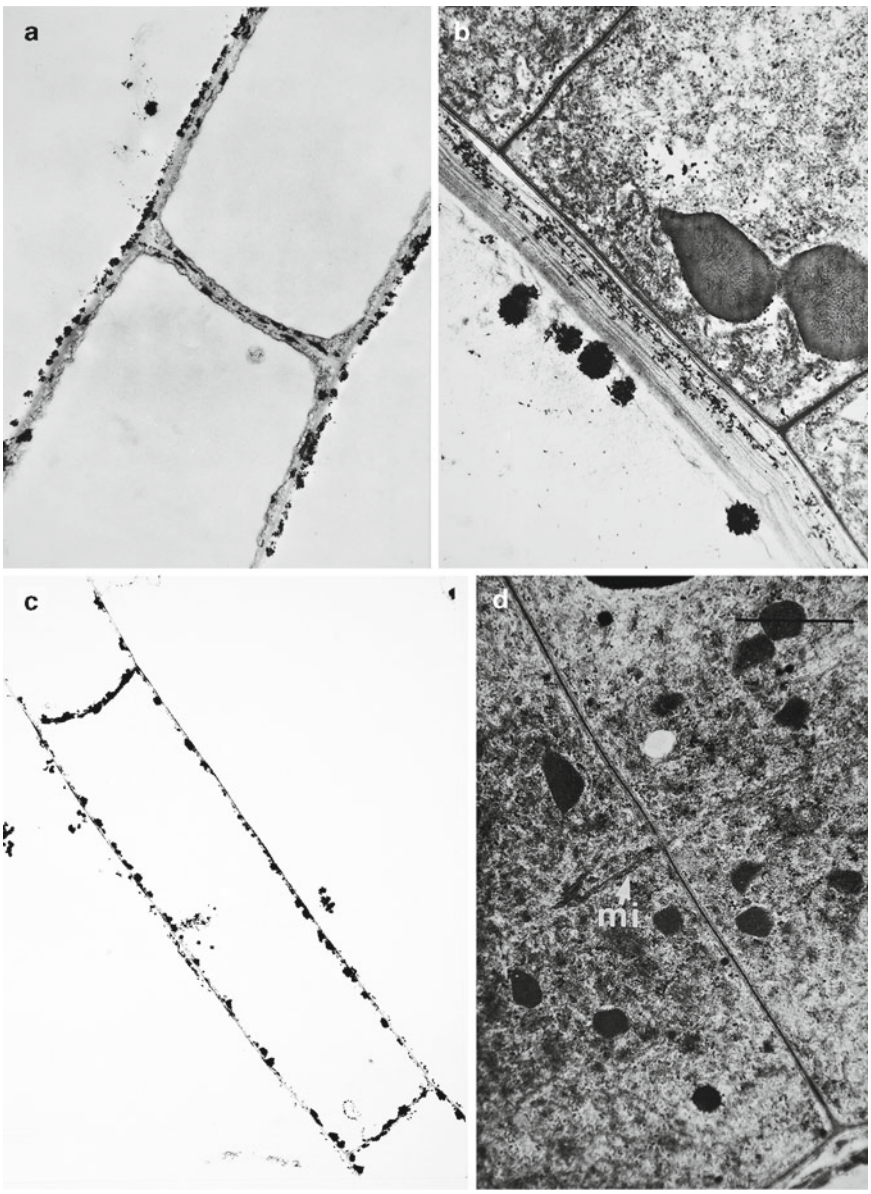

Fig. 22.8 (**a–c**) Examples of location of phosphomonoesterase (PMEase) activity following incubation in *para*-nitrophenyl phosphate (2 mg L^{-1} P) for 30 min with subsequent staining in 1% lead nitrate to deposit lead phosphate: (**a**) PMEase activity localized mainly on outer wall layer of the hair cells of *Calothrix parietina* D184 (= PCC 7713); (**b**) PMEase activity in sheath of *C. parietina* D184; (**c**) PMEase activity mostly inside the cytoplasmic membrane of hair cells of *Rivularia biasolettiana* from a stream in Middleton-in-Teesdale, UK; (**d**) Microtubule crossing middle of the cross-wall of a vegetative cell of *Calothrix parietina* D184: mi, microtubule (Photos A. Peat, P. Wood and B.A.W.)

species, growth stage and to show the influence of the environment. Light microscopy using BCIP (bromo-4-chloro-3-indolyl phosphate) and a simple phosphomonoester as substrate to generate a blue stain can provide much useful ecological information, but electron microscopy with precipitation of lead phosphate is needed to show the exact location of PMEase (Wood et al. 1986).

PMEase activity usually occurs on the outer layer of the cell wall (Fig. 22.8a); it is often especially strong on the cross-walls. In *C. parietina* strains it develops on all cell walls (apart from the heterocyst) and also in the sheath (Fig. 22.8b). It is more restricted in some other Rivulariaceae, developing mainly on the hair cells and chlorophyll-containing cells near the hair and also the part of the sheath furthest from the heterocyst. However, the extracellular matrix of *Rivularia* colonies often shows marked PMEase activity. It is uncertain whether PDEase activity is also present, but in view of the absence of soluble extracellular PDEase, this seems unlikely. Electron micrographs of old cultures of *Calothrix* and field *Rivularia* indicate that some activity also occurs inside the cytoplasmic membrane of old hair cells (Fig. 22.8c), suggesting that organic P molecules sometimes pass through the cytoplasmic membrane of the hair cell. Hairs of *Calothrix* D550 formed in response to Fe limitation show no phosphatase activity (Douglas et al. 1986).

In *C. viguieri* D253 (= PCC 7709) phosphatase activity is largely restricted to the hairs (Mahasneh et al. 1990). This strain, which had been isolated from a mangrove root, was able to grow in media of varying salinities ranging from freshwater to 20% seawater. However, when the culture was P-limited, hair formation only occurred in freshwater medium. During growth in saline medium the PMEase activity was

much lower and PDEase activity absent, while yields were less for most of the organic P substrates tested as the P source for growth. It would be interesting to establish whether such differences reflect adaptations to variations in the P status of water at its original location, such as might occur over a 14-day tidal cycle.

Once phosphate ions are released in the hair, rapid transfer must occur to the base near of the trichome where polyphosphate granule formation starts. Electron-microscopy of several strains showed conspicuous granule formation here within 1 min, but much later further along the trichome (Wood 1984). Studies are needed to establish the exact mechanism by which phosphate moves from the hair to the base of the trichome, but presumably a diffusion gradient is important. *Calothrix* D184 has a conspicuous microtubule passing through the middle of the cross-walls of vegetative cells (Wood 1984). This extended for about 1 μm each side of each cross-wall, but apparently not the whole length of the cell (Fig. 22.8d).

Several studies have permitted comparisons to be made between Rivulariaceae versus other cyanobacteria. In one of these (Whitton et al. 1991) this was based on the ability of 50 cyanobacterial strains (mostly tropical and subtropical) to grow in batch culture with various P sources (1 mg L^{-1} P) for 16 days; the study involved 30 Rivulariaceae and 20 non-Rivulariaceae (13 filamentous and 7 *Synechococcus*) and between hair-forming and non-hair-forming Rivulariaceae. Several differences were significant ($p<0.05$). In general *Nostoc* (six strains) produced the highest yields with inorganic P, whereas Rivulariaceae produced higher yields than filamentous non-Rivulariaceae with β-glycerophosphate, pNPP and DNA. All Rivulariaceae grew well with a phosphomonoester (pNPP, *para*-nitrophenyl phosphate) and a diester (bis-pNPP, bis-*para*-nitrophenyl phosphate) and all but one (*Calothrix* D764 from a deepwater rice field) grew with DNA. Many also used phytic acid (*myo*-inositol hexakisphosphate), but seven did not (including *Calothrix* D764). Rivulariaceae forming hairs were more effective than those not forming hairs at utilizing phytic acid. It seems likely that P is sometimes released by nuclease as well as phosphatase activity, since 10 strains, all Rivulariaceae, produced a yield with DNA at least 1.5 times that with pNPP.

This study also indicated that Rivulariaceae are especially sensitive to ATP. Eight Rivulariaceae (including six hair-formers) failed to show any growth with ATP (1 mg L^{-1} P) and in some cases this was toxic. All the other filamentous forms grew well with ATP. A possible explanation is that ATP enters the hair cells rather than being hydrolyzed at the surface and this leads to internal concentrations which are inhibitory or toxic.

The Rivulariaceae tended to show higher rates of surface and extracellular PMEase and surface PDEase activity and the highest rates all occurred in strains of *Gloeotrichia*; none of the strains in the study formed extracellular PDEase. Within the Rivulariaceae, strains from calcareous environments show higher PMEase activity than strains from non-calcareous environments at pH 10.3 ($p<0.01$), though not at pH 7.6. All strains in this study were assayed using 250 μM substrate, but a further study with 16 phototrophs showed that substrate concentration sometimes, but not always, influences the response to pH (Whitton et al. 2005; Whitton and Donaldson, unpublished data). The pH optimum for three *Calothrix* strains was 1.5–2 pH units lower using 1 μM substrate (methylumbelliferyl phosphate) compared with 250 μM; the lower pH value was close to the typical field pH All three strains were ones with hairs, as was the only eukaryote to show a similar response (*Stigeoclonium* D565). The strains not showing a response included all the Rivulariaceae not forming hairs. Perhaps the species showing two pH optima have more than one mechanism for mobilizing organic phosphate. Phytase activity has been reported for many Rivulariaceae, such as a *Calothrix parietina* strain from Upper Teesdale, UK, (Livingstone et al. 1983) and 35 of the 42 strains tested by Whitton et al. (1991: see above), but the influence of pH has yet to be studied.

Further insight into differences between Rivulariaceae and non-Rivulariaceae was provided by comparisons between two strains isolated from Rio Alberche, Spain: *Calothrix elenkinii* (not hair-former) and *Nostoc punctiforme* (Mateo et al. 2006). When inorganic phosphate was supplied to a P-limited culture, P uptake kinetics was closely similar for both strains. For instance, the half-saturation constant (K_m) was 2.98 μM for *C. elenkinii* and 2.99 μM for *Nostoc punctiforme*. This indicates that a higher affinity for a low inorganic phosphate concentration was not a factor likely to influence the relative success of *C. elenkinii*. The authors suggested that this was due to its greater ability to store P as polyphosphate granules and its higher surface PMEase activity. However, an *in situ* survey (Mateo et al. 2010) of phosphatase activities of *Rivularia biasolettiana* and three other cyanobacteria (*Schizothrix coriacea, Tolypothrix distorta* var. *penicillata* and *Nostoc verrucosum*) in the River Muga, found that *Tolypothrix* had the highest PMEase and PDEase activities (related to chlorophyll), *Schizothrix* the next highest and *Rivularia* the least on the four occasions measured. Surface phosphatase activities of all the populations obeyed apparent Michaelis-Mention kinetics, showing similar values for K_m, but markedly different ones for V_{max}, with *Rivularia* having the lowest values for both PMEase and PDEase activities. In March, when *Rivularia* not only showed the least phosphatase activities of any season, but also the greatest contrast with *Schizothrix* and *Tolypothrix* (*Nostoc* was absent), its trichomes had prominent polyphosphate granules. It was suggested that the contrasting results may be due to *Schizothrix* and *Tolypothrix* growing faster than *Rivularia* and already responding to the low ambient P found at the time.

Stream surveys provide records of *Calothrix* populations at zinc concentrations just below 10 mg L^{-1} (Shehata and Whitton 1981; Whitton et al. 1981), a value well above that for other heterocystous cyanobacteria. It is suggested that this is another example of an environment where an ability to use organic P effectively is important, because inorganic P is relatively insoluble under these conditions, especially as these streams also had elevated lead. Pb^{++} removal from wastewater by *Calothrix marchica* was higher in old than young material (Ruangsomboon et al. 2006), presumably because of increased sheath production.

22.4.3 Molecules with Possible Ecological Effects

A range of organic molecules with biological effects on cells of other organisms have been isolated from cyanobacteria (Smith and Doan 1999; Skulberg 2000; Van Wagoner et al. 2007), including some Rivulariaceae, such as the carbolines from *Dichothrix baueriana* (Larsen et al. 1994). In some cases the circumstantial evidence suggests that these may be involved in protection from grazing (see Sect. 22.6.3.3). In other cases, even when there have been no ecological studies, features of the molecule, including its effects in bioassays, suggest that it be well worth doing so. Schlegel et al. (1998) found that, of 198 cyanobacterial strains, 7 (out of 13) *Fischerella*, 5 (out of 28) *Nostoc* and 3 (out of 9) *Calothrix* had bioactivity against green algae; the *Calothrix* affected all three species tested. As the cyanobacteria were cultured in a very P-rich medium, it would be worth repeating the survey with old, P-limited cultures, especially for *Calothrix*. An extract of a marine *Rivularia* sp. showed marked antibacterial activity against gram negative bacteria in comparison with ampicillin and streptomycin (Zarmouh 2010). Studies initiated by the research group of G. D. Smith at Canberra were especially promising, so it is worth summarizing how this research proceeded.

This project started when Rickards et al. (1999) showed that cell extracts of two *Calothrix* isolates inhibited the growth *in vitro* of a chloroquine-resistant strain of the malaria parasite, *Plasmodium falciparum*, and of human HeLa cancer cells, both in a dose-dependent manner. Two pentacyclic metabolites (calothrixins A and B), with an indolo[3,2-*j*] phenanthridine ring system unique amongst natural products, were isolated and shown to have growth-inhibitory effects at nanomolar concentrations (Doan et al. 2000, 2001). Calothrixin A was one of two cyanobacterial molecules found to inhibit *Escherichia coli* RNA polymerase competitively with respect to ATP, and non-competitively with respect to UTP. Based on comparisons with the sensitivity of whole cells to these inhibitors, the authors concluded that other targets in addition to RNA polymerase may also be implicated in their action. The antiproliferative effect of calothrixins on several human cancer cell lines may be related to their ring structure (Chen et al. 2003) and their ability to undergo redox cycling (Bernardo et al. 2004); further insight to the mechanism was obtained by synthesizing related quinones (Bernardo et al. 2007). Among various effects on human cell metabolism, Khan et al. (2009) found that calothrixins act as poisons of DNA topoisomerase I and do so reversibly. The two molecules have now been synthesized (McErlean et al. 2007; Abe et al. 2011).

Among other molecules reported from Rivulariaceae, but few, if any, other cyanobacteria, are octadecatetraenoic acid (Kenyon et al. 1972), brominated phenolic substances from an axenic freshwater *Calothrix* and field material of marine *Rivularia* (Pedersen and DaSilva 1973) and six polybrominated biindole derivatives from *R. firma* (Norton and Wells 1982), the last being named rivularins by Maehr and Smallheer (1984). Several of these authors discussed possible biological effects, but apparently none has been tested thoroughly. In an account of gene products of cyanobacteria, Ehrenreich et al. (2005) listed a desert sand strain of *Calothrix*, which released a siderophore into the medium, as having some inhibitory effect on *Synechococcus* PCC 7002. Molassamide, a depsipeptide serine protease inhibitor was isolated from the marine *Dichothrix utahensis* (Gunasekera et al. 2010).

The possible role of microcystins and other toxins in reducing grazing is considered in Sect. 22.6.3.3.

22.5 Overview of the Genera

22.5.1 Introduction

The features of the five genera of Rivulariaceae were introduced in Sect. 22.2.1. The morphological features of these and other morphologically complex cyanobacteria frequently show an obvious relationship to features of their environment (Whitton 2008). It should therefore be possible to comment on the range of environments where Rivulariaceae occur from an understanding of their morphological diversity. In addition, the increasing realization of the molecular diversity of the genera (Sect. 22.3.2) raises the question as to how much it will be possible to recognize by morphological criteria the groups within the form-family Rivulariaceae which have close molecular similarity. If this proves possible, classical taxonomy could be revized to fit better with the molecular data.

The presence of heterocysts indicates that N_2 fixation is important at least some growth stage and thus there is probably a relative deficiency in combined N at that time. As the extent of tapering increases with increasing P limitation (Sect. 22.4.2), while hormogonia formation occurs when

phosphate is added to P-limited material, the hypothesis is suggested that the Rivulariaceae as a whole are adapted to environments showing alternating N and P limitation. The extent to which the available data supports this is discussed below.

22.5.2 *Calothrix*

In addition to trichome width, whether or not mature trichomes end in multicellular hairs and whether intercalary heterocysts form under some conditions, there are a number of features which vary within the genus. Some, but not all, have been used as criteria for distinguishing species. Two species have been reported to form akinetes. The original illustration of *C. stagnalis* by Gomont (1895) shows individual filaments epiphytic on *Cladophora*, so it is clearly not just a loosely organized *Gloeotrichia* colony; the trichomes have hairs.

The basal part of the trichome is swollen in some species (Sect. 22.2.2.1) and most, if not all, shown in Geitler (1932) are species forming hairs. The data by Sinclair and Whitton (1977a, b) together indicate that species with swollen bases tend to form hairs when P-limited. In the study of how Rivulariaceae strains respond to combined N (nitrate), all strains with swollen bases in N-free medium belonged to the group of strains where many trichomes retained obvious tapering (but no heterocyst) in the presence of N; none belonged to the other group where all the trichomes had parallel sides, much like *Lyngbya*. However, Rai et al. (1978) showed that the response of a *C. brevissima* strain differed according to N supply: slight tapering remained with NO_3-N, but not with NH_4-N. When a culture supplied with NH_4-N had depleted this, the trichomes formed intercalary heterocysts, followed by the initiation of polarity once more with trichome breakage adjacent to the heterocyst..

The other characters which have been incorporated into taxonomic descriptions are whether the sheaths are brown (scytonemin), whether the sheaths are layered (e.g. *C. parietina*) and whether there is a distinctive colony structure (e.g. *C. pulvinata*). There are thus at least six features in addition to dimensions that could be considered in relation to molecular groupings.

Batch culture studies on *Calothrix* D764, a strain isolated from a hormogonium in the plankton of a Bangladesh deep-water rice field provide insight on how trichome morphology, cell composition, nitrogenase and surface phosphatase activities interact (Islam and Whitton 1992a, b). Although this strain produced long, tapered filaments, which were ultimately very narrow and thus had the shape of a hair, the terminal cells remained photosynthetic and increased only slightly in length. Under P-rich conditions a hormogonium formed in the apical region of the trichome without any cell loss. Chlorophyll *a* ranged from 0.25% to 2.8% (dry weight), with values >1% only occurring for a short period when cellular P was at its maximum; 0.5% dry weight is probably typical for healthy field material. Chlorophyll a and phycocyanin contents both increased in% values as light intensity was reduced from 85 to 10 μmol photon m^{-1} s^{-1}. There was a shift of N from cyanophycin to phycocyanin formation with decreasing light intensity.

When P was added to a P-limited culture of *Calothrix* D764 at the highest light value, the maximum cellular P content was reached by 1 h, while the maximum nitrogenase activity by 1 day. After a relatively short period of very high nitrogenase activity, the rate per unit mass fell rapidly, being 150 times higher on day 2 than at 5 weeks. However, each heterocyst had to support about five times as many cells, so the equivalent comparison based on heterocysts between day 2 and 5 weeks was 32 times. Formation of new trichomes had slowed down by the end of the first week, but some still formed for a few more days; the subsequent increase in biomass resulted entirely from the filaments growing longer. Addition of P during this stage led to rapid formation of further hormogonia, each one being initiated at the end of the filament. The P content of 2.9% at day 1 fell rapidly and surface PMEase first became detectable when it had fallen to 0.95%. PDEase was initiated at the same time as PMEase and the two activities ran closely parallel for the first 2 weeks, with values for PDEase being about one-third those of PMEase. The presence of combined N in the medium led to higher surface PMEase and PDEase activities. Light had no effect on PMEase or PDEase activities on this or other *Calothrix* strains tested over periods up to at least 1 h, nor on two rice-field *Rivularia* strains (Banerjee and John 2005) or *Rivularia* colonies from River Muga (Mateo et al. 2010). This species grew almost as rapidly with glucose-6-P as with inorganic P and quite rapidly with phosphomonesters, but not with DNA, in spite of the high PDEase activity and the fact that most other *Calothrix* strains grow well with DNA as the P source (Whitton et al. 1991).

No doubt studies on other strains would all show slight differences in the sequence of events, but the morphological changes in *C. parietina* D550, isolated from a UK upland stream, were largely the same (Livingstone and Whitton 1983; Sect. 22.4.2), the main differences being that it formed colourless hairs, which involved cell wastage when hormogonium formation was initiated beneath it; in addition, hormogonium formation ceased at an earlier growth stage than in *Calothrix* D764. The key changes in batch culture of *Calothrix* seem clear. Nitrogenase activity is at its highest when the P content is highest; surface phosphatase activities are absent at this stage, but then start to increase quite rapidly. Shortly after this, hormogonia formation ceases, and only recommences if further P is added to the culture. Strains probably differ in that differentiation of hormogonia

Fig. 22.9 *Rivularia bullata* colonies on boulder taken from shallow water in the very slightly brackish Loch Àirdh a' Mhuile, South Uist, Outer Hebrides (Scotland), in September 2009 (Photo B.A.W.)

Fig. 22.10 Colonies of *Rivularia* cf *R. biasolettiana* and the red alga *Chroothece* growing in shallow water on the same boulder in a fast-flowing stretch of the Rio Chícamo, S-E. Spain. The dark colour of the *Rivularia* colonies is due to highly pigmented brown sheaths, while the orange-brown of the *Chroothece* is due to intracellular carotenoids (M. Aboal, personal communication) (Photo B.A.W.)

tends to cease earlier in strains forming hairs than in those which do not.

As about 100 *Calothrix* species have been described, it is not surprizing they are recorded from a range of environments. However, quite a number occur in highly variable ones, such as the upper part of the marine intertidal zone. *C. parietina* often forms part of the microbial community in the zone where the water level fluctuates in about half the mature garden ponds in the UK with water above pH 7.0 (B.A.W., unpublished). This also applies to *C. parietina* D550, which was isolated from the side of one of the streams described by Livingstone and Whitton (1984), where the ambient P concentration is highly variable and mostly organic. Organic P formed well over half the mean value for filterable P (n = 53) in Bangladesh deepwater rice fields (Whitton et al. 1988a), where *Calothrix* and *Gloeotrichia* were frequent, and where *Calothrix* D764 (described above) was isolated. In the case of the *Calothrix* population in Hunter's Hot Spring described by Castenholz (1973; Sect. 3.2.3.2; see also Fig. 3.2d, g), it seems probable that the intense grazing of the *Oscillatoria* mat immediately upstream releases organic P.

22.5.3 *Rivularia*, *Dichothrix* and *Isactis*

Rivularia species in classical floras are separated into marine and freshwater, though records are often confused because of freshwater streams flowing into the intertidal zone. Conversely, waterbodies near the sea, but which are only very slightly brackish, may have marine species, as shown for *R. bullata* (Fig. 22.9). The characters used for separating species are mostly similar in marine and freshwater forms: trichome width, maximum colony size, soft or firm, whether the sheath is layered, whether the ends of the sheaths are spread out in many layers; freshwater forms are also separated according to whether they show calcification. The taxonomic characters used for *Dichothrix* are similar to those for *Rivularia*, except that the extent to which hormogonia are arranged in bundles is important; there is a tendency for the trichomes of marine species to be wider than freshwater species. *Isactis* is unispecific; it is little more than a large group of vertical *Calothrix* filaments embedded in mucilage.

Freshwater *Calothrix* and *Dichothrix* occur in both very soft (Heuff and Horkan 1984) and hard waters. The records for Czech and Slovak Republics listed by Skácelová (2006) confirm this for *Dichothrix*. Most records for *Rivularia* are for calcareous waters (Fig. 22.10) and it is often the dominant near the source of highly calcareous streams (see Whitton 1987; Sabater 1989; Sabater et al. 2000). However, in Jämtland, Sweden, and probably elsewhere in northern Scandinavia (Johansson 1979; Johansson, personal communication 1981) the genus is widespread in non-calcareous waters. In the marine environment *Dichothrix* is frequent on submerged angiosperms (Uku et al. 2007) and floating masses of larger algae. Floating masses of *Sargassum* and epiphytic *Dichothrix fucicola* contributed over 0.5% total primary production in the western Sargasso Sea (Carpenter and Cox 1974), while production in October by *Dichothrix* was about 15% macroalgal production in continental shelf waters, though only 1.2% in the northern Sargasso Sea. The greater abundance of *Dichothrix* in shelf waters than the Sargasso Sea probably reflects the availability of Fe. *Calothrix* is also frequent on marine angiosperms, but more especially on ones that are intermittently exposed, such as described by Webber (1967).

The cycle of morphological changes for *Rivularia* in relation to ambient P is similar to that for *Calothrix*, but the periodicity of hormogonia formation and release is much longer. Streams in Upper Teesdale, UK, show an annual cycle of elevated P in spring, followed by far lower concentrations for much of the rest of the year (Turner et al. 2003a, b). The annual cycle of changes was especially clear in 1981–1982 (Livingstone and Whitton 1984); most release of hormogonia by *R. biasolettiana* occurred in early spring following a period of high organic P concentrations in the water. However, in some years there are further short periods of sufficiently elevated P concentrations to lead to marked hormogonia release and further colony formation (Whitton et al. 1998). In addition to the release of hormogonia, trichomes arising from "false" branches occur in almost all Rivulariaceae; the lower part of the original trichome develops a tapered shape as it grows. However, in some cases true hormogonia are formed which are not released from the colony, but differentiate into typical filaments inside the colony.

Although growth of an upper intertidal *R. atra* population at Tyne Sands, S-E. Scotland, occurred mostly in summer (Yelloly and Whitton 1996), periodicity of colony formation depended on a combination of extreme storm events and tides. Storms deposited large masses of detached, mostly sublittoral, seaweeds high on the supralittoral. These rotted and released high concentrations of nutrients next time there was a high spring tide; much of the filterable P was organic. All the trichomes in large colonies released hormogonia within a couple of days, leading to the formation of new colonies.

Dichothrix shows a similar response to periods of elevated ambient P, with hormogonia release followed by colony formation, but the periodicity seems less clear-cut. *Dichothrix* shows a marked tendency to be more frequent than *Rivularia* in the west of the British Isles, while the reverse applies in the east (Whitton 2011). It was suggested that this may be favoured by the greater and more irregular precipitation and less marked seasonal difference between summer and winter that occur in the west. These may also explain why the dominant *Rivularia* species in streams on Clare Island off the west coast of Ireland is *R. beccariana* (Whitton 2007), which forms only very small colonies, and probably does so for much of the year rather than mainly in spring.

Many studies have reported that *Calothrix* species and sometimes Rivulariaceae in general are associated with low levels of nutrients. For instance, Schneider and Lindstrøm (2011) presented an index based on non-diatomaceous benthic cyanobacteria and algae to characterize river trophic status in Norway based on total ambient P; all the Rivulariaceae showed a low indicator value. Although such indices are often required by water administration organizations, they neglect the importance of temporal variability in flowing waters draining semi-natural ecosystems and may hinder understanding of the processes occurring there.

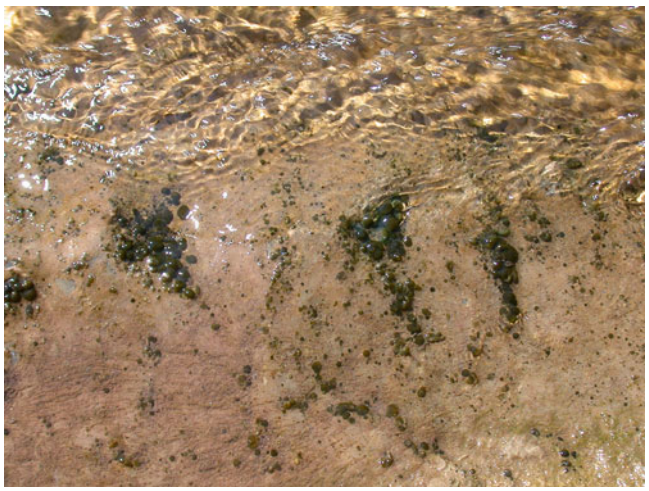

Fig. 22.11 *Rivularia* cf *R. biasolettiana* in River Muga, N-E. Spain, showing range of colony size reflecting older colonies and recently formed ones (Photo P.M.)

Many "oligotrophic" waters do not contain Rivulariaceae. However, the sites with *Rivularia* studied by Livingstone and Whitton (1984) and Yelloly and Whitton (1996) do at times have high concentrations of ambient P (in some cases >1 mg L^{-1} P for short periods), even though the annual mean is far lower.

It is unclear how wide a range of P concentrations or N:P ratios is required to provide competitive success for Rivulariaceae and whether an alternation between low and extremely low values would be suitable. The River Muga, Spanish Pyrenees, never showed values for ambient total filterable P > 6.8 μg L^{-1} P (mostly organic) during measurements at five different times of year (Mateo et al. 2010), yet new colonies can form in spring (Fig. 22.9).

The *Rivularia* population in the highly calcareous and slightly saline Rio Chicamo, S-E. Spain, presents a still unexplained anomaly (M. Aboal, personal communication). Although healthy colonies with typical heterocysts are abundant in some stretches of the river, often together with the red alga *Chroothece* (Fig. 22.11), the mean concentration of ambient combined N is high, hence no obvious advantage for a N_2-fixer. However, the mean P concentration is low and mostly organic, features which are likely to favour *Rivularia*. The most probable explanation is that there is some season of the year or river condition when combined N is sufficiently low to give *Rivularia* a selective advantage in competition with *Chroothece*, but this has yet to be established. Other possible (though unlikely) explanations for formation of the hormogonia is that at some time of year *Rivularia* switches almost entirely to P accumulation, leading to a high internal concentration in spite of low ambient concentration, or that some other element shifts from scarcity to abundance for a short period and this triggers hormogonia formation.

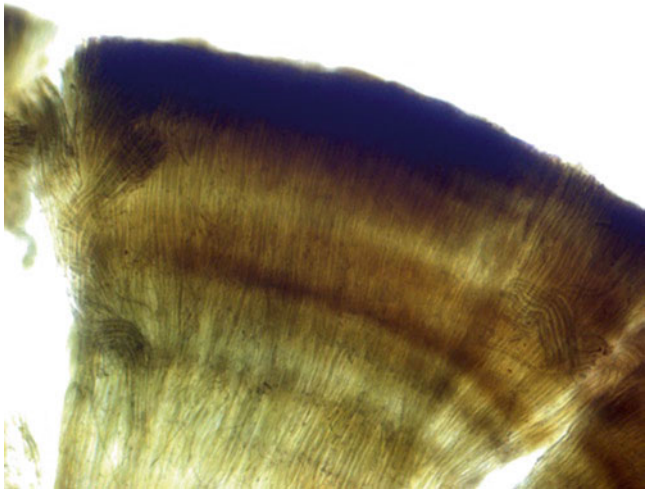

Fig. 22.12 *Rivularia haematites*, from R. Guadiela, Spain. The layers of calcite obvious in field material have been dissolved with very dilute acid, but a banded structure is still obvious due to differences in sheath density and scytonemin formation (Photo P.M.)

The seasonality of growth of *Rivularia* in many temperate region streams and lake margins suggests this may be an important factor leading to the laminated structure of many colonies, especially those treated as *R. haematites* (Fig. 22.12); evidence for this in a UK stream population was given by Pentecost (1987). Intermittent exposure to the air and evaporation during the warmer season led to some calcium carbonate deposition in this population. *Rivularia* EPS may also trap and bind calcite crystals (Pentecost and Riding 1986). The factors influencing calcite formation inside *R. haematites* colonies were studied at five sites in the Salzkammergut, Austria, by Obenlüneschloss and Schneider (1991). The sites provided a wide range of conditions for light, flow variability and current speed and calcite crystal size showed an obvious correlation with these. For instance, the largest and most regular crystals occurred at sunny sites with continuous flow and low current speed. The use of planar optodes and microelectrodes helped Pentecost and Franke (2010) to study the process of calcification in still more detail. A quantitative assessment of the factors involved at each of various depths within the colonies of two populations (*R. haematites* and *R. biasolettiana*) led them to conclude that about 14% total calcium carbonate was the result of photosynthesis. The calcite crystals grow parallel to the trichomes and nucleate on the fibrous outer layer of the sheath (Caudwell et al. 2001).

The formation of laminae in *R. haematites* was monitored over a 7-year period by Caudwell et al. (1997, 2001) in central France. A micritic dark lamina formed in three stages: (i) initial development of a dark lamina in the zone where the filaments develop false branching during the wet season; (ii) calcification in this zone as microsparitic and sparitic lamellae during dry spells; subsequent bacterial micritization of the lamellae during an extended warm dry season. In the particular climate experienced during the study period there was either a single dark lamina for 2 years growth or a dark lamina thicker than the annual growth rate. This suggests that the extent to which laminations reflect marked annual changes in climate and nutrient concentrations is likely to differ between regions and sometimes between years. Although carbonate deposition is rare among marine *Rivularia*, it has been reported for *R. mesenterica* and *R. polyotis* (Golubic and Campbell 1981), but apparently without laminations. In a comparison (Myshrall et al. 2010) of stromatolitic and thrombolitic (non-laminated) mats at Highbourne Cay, Bahamas, the most obvious difference was the button mats of calcifying *Dichothrix* in the latter. *Dichothrix* can also be an important component of freshwater calcifying cyanobacterial communities (Whitton et al. 1986), individual colonies are not large enough to form distinct layers. Its role in the marine environment is discussed in Sect. 22.7.

Two other genera have been described. *Sacconema* Borzi (1882) ex Bornet et Flahault (1886) seems little more than a form of *Gloeotrichia* with very thick and partly confluent sheaths, while *Gardnerula* (de Toni 1936) resembles a large *Dichothrix* colony with subdichotomous branching. Both genera were mentioned briefly by Castenholz (1989), but only *Gardnerula* by Rippka et al. (2001c). However there seems little reason why these should be treated as separate genera rather than species of *Gloeotrichia* and *Dichothrix*, respectively.

22.5.4 *Gloeotrichia*

Gloeotrichia includes all the colonial forms producing akinetes. Most are benthic in shallow lakes, ponds and ditches, but *G. raciborskii* and *G. natans* typically float at the surface for a considerable period, *G. pisum* and similar forms are epiphytic on older parts of submerged aquatic plants and *G. echinulata* is planktonic during part of its growth cycle. *G. ghosei* was reported as floating or benthic (Claassen 1973) and it seems likely that some other species are the same. Other characters used in species descriptions include size of colonies, whether the sheath is layered, whether the hair extends beyond the surface of the colony and the size and shape of akinetes. Akinete details are in some cases essential for species recognition, while long inspection of a pond population may be needed to decide whether or not it can form akinetes. If not, it would be recorded as *Rivularia*, as molecular data would be needed to exclude the possibility that it was a *Gloeotrichia* which had adapted for survival at that site by no longer forming akinetes..

Akinete formation often leads to disintegration of the trichome. However, sometimes several akinetes are formed,

whilst at other times the trichome breaks away and can then form another akinete. Many queries could be answered by careful observation. To what extent is the behaviour of the trichome subsequent to formation of its first akinete a species- or strain- specific character? To what extent can increase in colony number occur without akinete formation? Does the large size of the akinete permit a colony to develop from a single trichome and, even if so, does colony formation normally involves trichome aggregation. If trichome aggregation is the norm for colony initiation, what happens if the trichomes are too sparse for this to happen? The highly tapered and highly gas-vacuolate trichomes lacking a heterocyst which were occasional in the plankton of a shallow lakes on Clare Island in 2004 may have been such a case (Whitton 2007). These might have been a residual population of *G. echinulata* too sparse to permit trichome aggregation, as this species had been reported on the island by West (1912), but typical material could no longer be found there.

Apart from *G. echinulata, G. natans* is the most widely reported species, though apparently more frequent in subtropical and tropical waters. It is common in rice fields. For instance, it is the most widespread cyanobacterium in rice fields in Chile (Pereira et al. 2005), frequent in rice fields around Los Baños, Philippines (Martinez-Goss and Whitton, unpublished data) and frequent in deepwater rice fields near Manikganj, Bangladesh (Rother et al. 1988). Populations usually start to develop as attached colonies, but then become detached; these floating colonies sometimes become intermingled with submerged macrophytes, but often they float to the surface. Young colonies may include trichomes with gas vacuoles (Geitler 1932), but old colonies without gas vacuoles may still float.

A Philippines rice-field isolate was studied in the laboratory and outdoor raceway ponds by Querijero-Palacpac et al. (1990), but BG-11_0 medium was used, so the results are unlikely to reflect its behaviour in nature. In the laboratory the specific growth rate was 0.076 h^{-1}. Using a stirring rate of 30 rpm in the raceway ponds, daily production of cultures harvested to maintain cell densities of 0.7, 1.15 and 1.5 g (d. wt) L^{-1} was 24.7, 17.1 and 18.1 $g\,m^{-2}\,day^{-1}$, respectively. The phycobiliprotein content in the culture maintained at 1.5 $g\,L^{-1}$ reached 14% of the biomass.

The planktonic *G. echinulata* has received considerable study. Some of the first field experiments were by Spodniewska (1971) using suspended bottles during summer in the eutrophic, holomictic Lake Mikołasjskie, Poland. She found that respiration averaged about 30% gross primary production for *G. echinulata.* A suggestion by Rodhe (1949) that *G. echinulata* needed an unknown organic component for growth led Zehnder (1963) to obtain evidence that the only organic factor required for laboratory growth is a chelating agent. Lange (1974) reported that *G. echinulata* was one of four bacterized strains (species) of cyanobacteria which failed to grow without added chelator, as opposed to six which did. However, even when an artificial chelator was included in the medium, Chang (1979a) found that soil extract and 5–40 mM glucose both aided growth of an axenic strain. It has still not been established clearly whether or not planktonic *G. echinulata* forms its own chelator. This also raises the question as to how important accumulation of elements besides P (see below) is for colonies developing on bottom sediments.

Although many of the reports of conspicuous *G. echinulata* populations are for lakes which might otherwise be considered oligo- to mesotrophic, research was focussed initially on eutrophic lakes. Caution is needed in comparing results for a bloom-forming species at different sites, as has become clear in the study of *Microcystis* (Chap. 7). Formation of dense plankton populations of *Gloeotrichia echinulata* was first described for Ellesmere, a shallow English lake, by Phillips (1884), though he quotes an 1880 report which mentioned its period of hibernation at the bottom and then rising in summer. However, the first detailed account was by Roelofs and Oglesby (1970) for Green Lake, Washington, a shallow lake without a permanent thermocline in summer. Subsequent to *G. echinulata* disappearing from the plankton in September, a bottom sample taken in November showed akinetes, but no colonies. However, developing colonies were found on the bottom in the following January. The authors mentioned short filaments of 4–6 cells, though they did not explain whether these were organized into spherical colonies. Colonies were still absent from the plankton on 23 June, but were entering it on 3 July. Apparently colonies were developing on the bottom for 6 months, while the planktonic phase only lasted for about one-quarter of the year. In a 2-year study on the same eutrophic lake, Barbiero and Welch (1992) concluded that the plankton derived 40% *G. echinulata* colonies from the benthos and that these accounted for a significant part of the internal P loading of the lake. However, Istvánovics (2008) concluded from their results and those of Karlsson-Elfgren et al. (2003) that recruitment of colonies was insignificant in some years and so the internal P load is highly variable in *Gloeotrichia* lakes.

The most detailed studies have been on the moderately eutrophic, stratified (in summer) Lake Erken in S-E. Sweden. Heavy blooms of *G. echinulata* often occur during July and August at the same time as the epilimnion deepens gradually to 10 m (Pettersson et al. 1990, 1993). Like other wind-exposed lakes, akinetes and colonies are found in large numbers on the bottom sediments (Istvánovics et al. 1993). Benthic colonies averaged 5×10^5 m^{-2} in the top 4 cm of sediments in areas of the lake shallower than 10 m in March 1991 (Pettersson et al. 1993). Istvánovics et al. (1990) and Pettersson et al. (1990) suggested that epilimnetic growth might be largely or solely based on the internal P pool obtained while the organism was associated with the sediments and a number of P uptake experiments led Istvánovics et al. (1993)

to conclude the colonies were unable to acquire any P in the epilimnion. The P uptake threshold exceeded the epilimnetic concentration of soluble (filterable) reactive P by an order of magnitude. An overview of the results for Lake Erken indicated that recruiting colonies translocated some two-thirds of the total net P load from the sediments to the epilimnion (Istvánovics 2008). It is not clear how this fits with the observations of Karlsson-Elfgren et al. (2003) made during a 2-year study that recruitment only contributed to <5% of the maximum *G. echinulata* abundance during late summer. However, the authors emphasized that variations in the measured abundance of *G. echinulata* could reflect measured rates of migration from the sediment, and variations in either pelagic colony division rate and pelagic residence time.

The conclusion of Istvánovics et al. (1993) that epilimnetic *G. echinulata* did not use organic P in Lake Erken was based on "alkaline phosphatase" activity measurements using the method of Pettersson (1980). However, this methodology has limitations. Because of the high substrate concentration used, the results may be unreliable at the pH tested (Whitton et al. 2005). PMEase activity tends to be higher in Rivulariaceae than other cyanobacteria (Sect. 22.4.2) and when PDEase activity was compared, a *Gloeotrichia* strain showed the highest ratio to phosphomonoesterase activity to any strain, suggesting the importance of phosphodiesters as substrates (Whitton et al. 1991). In addition *Gloeotrichia* strains were the most successful genus at utilizing phytic acid. Although these strains did not include *G. echinulata*, they indicate the importance of checking its ability to use a range or organic P compounds under realistic assay conditions. If the hairs of planktonic *Gloeotrichia* do not have this role, are they little more than a relic of structures important at a late stage of benthic growth which helps to enlarge colony size?

The possibility that iron and boron might influence growth of *G. echinulata* colonies in Lake Erken was considered by Hyenstrand et al. (2001) using an *in situ* experiment. Iron had the most effect, enhancing the effect of adding phosphate and nitrate. Addition of boron led to an even greater increase, but boron had no effect if there was not additional iron at the same time. Studies by Vuorio et al. (2009) on carbon isotope signatures in colonies in Erken and Pyhäjärvi (Finland) showed that there was a systematic increase in $\delta^{13}C$ with increasing colony size, in spite of substantial differences in the average $\delta^{13}C$ in the two lakes. The response to size was probably due to diffusion limitation of C availability.

Carey et al. (2008, 2009) reported on recent outbreaks of *G. echinulata* in oligo- to mesotrophic lakes in N-E. USA, where at least 27 examples had occurred between 2002 and 2006 in Maine and New Hampshire. In one of these, Lake Sunapee, recruitment of colonies was 1.13 and 0.32 colonies cm^{-2} day^{-1} in 2005 and 2006, respectively. These values contrast with the 36 colonies cm^{-2} day^{-1} for Green Lake (Barbiero 1993) and *c.* 1,520 colonies cm^{-2} day^{-1} for Lake Erken (Forsell and Pettersson 1995). Nevertheless Carey et al. (2009) concluded that increased sediment total dissolved phosphate may have influenced recruitment. A pulse of sediment was typically followed by an increase in *G. echinulata* 18–19 days later. The authors commented that this time-lag corresponds to the time akinetes need to germinate (1–7 days) and take up P from the sediment (2–3 weeks) before recruiting to the water column (Tymowski and Duthie 2000; Karlsson 2003). Carey et al. (2009) also raised the question as to whether the influence of P on germination is accelerated by a discrete pulse or because the concentration exceeds a particular threshold, as suggested by Pettersson et al. (1993). Temperature and light are also factors which can be involved (Karlsson-Elfgren et al. 2004).

In spite of the many studies on *G. echinulata* the growth and division of colonies have not been described in detail, though partially separated, healthy colonies quite often occur in samples. Grazers can impact in various ways, including removal of a whole colony or disruption into fragments (Sect. 22.6.3.1). It is unclear whether single filaments or the small groups remaining after grazing can continue growth and perhaps eventually form akinetes. *G. echinulata* can also have a considerable impact on other phytoplankton grazers. Colonies and culture filtrate of Lake Erken *G. echinulata* both stimulated the growth of some species known to co-occur with it in Lake Sunapee and Lake Erken (Carey and Rengefors 2010). Of the seven species tested, none isolated from Lake Erken, five were stimulated by the presence of colonies and two by filtrate. The potential effects of zooplankton populations may depend on the duration and severity of a bloom and the tolerance of the zooplankton to potential toxins; zooplankton populations may be able to maintain themselves during prolonged blooms (Fey et al. 2010). However, interactions with *G. echinulata* and zooplankton might change with time due to the development of tolerance to toxins. Cyanobacteria and heterotrophic bacteria associated with *G. echinulata* colonies in Lake Erken, Sweden, may be a supplementary food source for the grazing zooplankton (Eiler et al. 2006), especially *Daphnia pulex* (Fey et al. 2010).

The use of experimental ponds by Carey et al. (2011) showed that in nutrient-limited ponds the presence of *G. echinulata* led to an increase in the biomass of small-sized phytoplankton and that this stimulation was related to the zooplankton biomass. An increased zooplankton biomass led to increased grazing of colonies, which may have increased the rate of nutrient leakage to other phytoplankton, thereby intensifying the stimulatory effect. In contrast, *G. echinulata* had a negative effect on small-sized phytoplankton in eutrophic ponds. The authors concluded that in nutrient-limited systems, *G. echinulata* may subsidize plankton food webs in nutrient-limited systems through nutrient leakage and could thus accelerate eutrophication.

In several lakes studied in the UK, such as Talkin Tarn, Cumbria, akinetes in planktonic colonies are most frequent towards the end of the growth period, though occasionally occur earlier in the season (B.A.W., unpublished). The storage granules abundant in the akinetes are cyanophycin, suggesting the trichomes are P-limited by the time the akinetes form; presumably they are important as a N source when germinating on the sediments. P limitation is therefore one factor likely to be involved, but other factors should be investigated such as possible responses to grazer activity.

In view of the many studies on *G. echinulata* we will summarize our own interpretation of the results. The species can occur in lakes or other waterbodies ranging from near oligotrophic to eutrophic, but obtains much of its phosphate from bottom sediments. It is therefore possible for moderate blooms to occur in lakes where water management organizations had not expected a problem, providing the sediments are rich enough in P. The presence of the organism may itself enhance eutrophication over a number of years. Although no evidence of P uptake could be found for epilimnetic *G. echinulata* in Lake Erken, further research is needed before it can be assumed that this applies to most lakes. The possibility that hairs have a role in mobilizing organic P in less nutrient-rich lakes should be investigated.

Populations can persist in lakes ranging over at least three orders of magnitude of colony recruitment from the sediments. The species mostly occurs in stratified lakes, but populations can persist in relatively shallow unstratified lakes, at least for a few years. In temperate regions it persists on the bottom for the majority of the year, but little is known about how much growth occurs during this period. Although the species occurs in the tropics (e.g. Aldabra Atoll, 9° S: Donaldson and Whitton 1977; central Brazil, 15° S: Campos and Senna 1988), there are no accounts of its seasonality in large tropical waterbodies.

22.6 Interactions with Other Organisms

22.6.1 Free-Living Phototrophs

There are many records of *Calothrix* and *Gloeotrichia*, and occasionally other Rivulariaceae, growing as epiphytes, but the extent to which this depends on features of the host plant or merely the overall chemical environment is usually unclear. However, the physical association with the host plant is especially close in some cases, such as the occurrence of *Calothrix* and *Gloeotrichia* (and several other cyanobacterial genera) on the lower epidermis and reproductive pockets of *Lemna* leaves, and are presumably responsible for the N_2-fixing activity associated with some duckweed blooms (Duong and Tiedje 1985). A small colonial *Gloeotrichia* is frequent on the submerged stems of deepwater rice plants in Bangladesh at situations where the underwater parts of the plant are well illuminated, such as next to channels (Whitton et al. 1988b).

There are many studies on the effects of cyanobacteria on rice plants, but most have used strains which had not been growing in the vicinity of rice plants in the field. However, Karthikeyan et al. (2009) studied the effects of 8 axenic strains, including 3 *Calothrix*, which had been isolated from the rhizosphere of a wheat cultivar. *C. ghosei* showed the second highest nitrogenase activity in the light and the highest value in the dark. When wheat seedlings were co-cultured with *C. ghosei*, short filaments were found inside the root hairs and cortical region. Such strains were considered promising candidates for developing plant growth-promoting associations with wheat.

In a comparison of the distribution of the epiphytes on the marine angiosperm *Posidonia* by Trautman and Borowitzka (1999), *Calothrix*, the only cyanobacterium included in the list, grew on both sides of the leaves of *Posidonia australis*, though restricted to the basal sections. Other records include on *Cymodocea nodosa* (Reyes and Sansón 1997) and on *Thalassia testudinu*m, where N_2-fixing activity was correlated with the abundance of *Calothrix* (Capone and Taylor 1977). A similar correlation was suggested for N_2-fixing activity on the mangrove *Avicennia marina* (Hicks and Silvester 1985).

Colonial Rivulariaceae almost always contain other cyanobacteria and sometimes eukaryotic algae as well, especially pennate diatoms. Perhaps the initial period of trichome aggregation leading to colony formation permits entry of some other motile species, though little is known about the extent to which invasion of mature healthy colonies occurs. Chang (1983) reported that *Pseudanabaena catenata* was frequent in *Gloeotrichia echinulata* colonies in Plöner See, Germany, in August and September, but not in *G. echinulata* colonies earlier in the year. Based on various observations, the author suggested that release of substances by *G. echinulata* may favour growth of *Pseudanabaena catenata.*

Quantitative studies on the frequency of various types of diatoms in and on colonies of the marine *Rivularia atra* versus their frequency as epiphytes on macroalgae were made by Snoeijs and Murasi (2004); the former relationship was described as symbiosis, but this extends the definition well beyond that used by most authors. Among the 35 most frequent diatom species, the relative abundance of species with motile and epipsammic life-form was 2.5 times higher within the *Rivularia* colonies than those with the attached epiphytic life-form. The frequency of epipsammic forms in the colonies was due to the frequency with which sand grains were incorporated into the colonies. Diatom community diversity was higher in the *Rivularia* samples, probably because cells epiphytic on the colonies were included. The motile diatoms inside the colonies were ones that occurred in mucilage matrices when present on stones. Other reasons were suggested why the

relationship should be favourable for diatoms, including the fact that the colonies are tough and can resist wave action; the authors did not consider the possibility that *Rivularia* toxicity might deter grazers (see below), nor did they have any suggestion as to how *Rivularia* might gain from the interaction, other than some possible nutritional advantage.

22.6.2 Symbiotic Associations

Extracellular growths of *Calothrix* have been reported from the thalli of several marine green algae, either entirely within the tissue, as in the *Enteromorpha* reported by Lami and Meslin (1959) and *Codium decorticum* from USA (Rosenberg and Paerl 1981), or partially epiphytic and partially inside the tissues, as a *Codium* from New Zealand (Dromgoole et al. 1978). In the case of *C. decorticum, Calothrix* was one of three cyanobacteria responsible for nitrogenase activity within a reducing microzone.

The best known symbiotic associations between *Calothrix* and other organisms are cyanobiont-containing lichens (Adams 2000). Although *Nostoc* is much the most frequent cyanobiont in lichens, *Calothrix* is probably the next most frequent. According to Ahmadjian (1967), it is the phototroph in *Lichina*, *Calotrichopsis* and *Porocyphus* and *Dichothrix* in *Placynthium*, although other authors consider the phototroph of some *Lichina* corresponds better to *Dichothrix*. The morphological appearance inside the lichen usually has only slight resemblance to free-living *Calothrix*. Henssen (1969) found that the phototroph of *Lichina rosulans* consisted of short, contorted chains and basal heterocysts were rare. Basal heterocysts, but no tapering were found in *L. polycarpa*, while the photobiont of *L. tasmanica* and *L. willeyi* had normal tapering (Henssen 1973). An ultrastructural study of *L. confinis* by Janson et al. (1993) showed that heterocysts were present in the thallus, but differed from the rest of the *Calothrix* cells in that no haustoria were in contact with them. Glutamine synthetase levels in the cyanobiont were a great deal less in free-living (cultured) *Calothrix* sp. The decrease was greater in mature parts of the lichen thallus than in the apical region. Rubisco was mostly located in carboxysomes of the vegetative cells of the cyanobiont. In free-living (cultured) *Calothrix* sp., phycoerythrin was located along the thylakoid membranes in both vegetative cells and heterocysts. Although the cyanobiont cells showed a similar pattern, the levels were lower.

The cyanobacterium in the coralloid roots of the cycad *Encephalartos hildebrandtii* showed no resemblance to any well known genus, but developed what the authors considered to be a typical *Calothrix* morphology in culture (Huang and Grobbelaar 1989). The micrographs of the association do not show unequivocally that the organism is *Calothrix*, perhaps because the study used BG-11$_o$ medium, so further studies are needed. Although the endophyte exhibited substantial nitrogenase activity, no heterocysts could be distinguished, yet heterocysts were found during culture; akinetes were also formed, again raising doubt whether this symbiont really is *Calothrix*. Thajuddin et al. (2010) reported one strain of *Calothrix* (again based on culture with BG-11$_o$ medium) among 18 cyanobacteria from coralloid roots of *Cycas revoluta;* presumably they overlooked the previous study, as they claimed their record to be the first for *Calothrix* as a cycad symbiont.

The importance of marine diatoms with symbiotic N_2-fixing cyanobacteria in various subtropical oceans, especially the North Pacific, has only become clear in recent years, although they were first described by Lemmermann (1905). The two intracellular symbionts, *Richelia intracellularis* and *Calothrix rhizosoleniae*, both have terminal heterocysts, but only the latter has a trichome with obvious tapering. Although *Richelia intracellularis* has not been isolated and brought into long-term culture, this has been done for *Calothrix rhizosoleniae* from *Chaetoceros* in the N. Pacific (Foster et al. 2010). The trichomes retained the terminal heterocyst, but 2 years after isolation a culture also showed intercalary heterocysts. The *nifH*, 16S rRNA and *hetR* sequences were amplified and cloned from this isolate and field populations of *Richelia* associated with *Hemiaulus hauckii* (N. Atlantic) and *Rhizosolenia clevei* (N. Pacific) (Foster and Zehr 2006). The results indicated that the symbionts in the three different hosts are distinct species or strains. Based on assays of *nifH* gene abundance and gene expression of plankton populations, there was no record for *Calothrix rhizosoleniae* in the western tropical Atlantic (Foster et al. 2007), but there was in the eastern equatorial Atlantic (Foster et al. 2009). This was probably the intracellular symbiont, but the possibility of free-living trichomes could not be ruled out.

A comparison of the growth rates of cells in symbiotic association with estimates for ones which were not showed that the rates were higher for symbiotic cells (Foster et al. 2011). In the case of N_2 fixation, the rates were similar among the symbioses and there was rapid transfer (within 30 min) of fixed N. The N_2 fixation rates estimated for *Calothrix* and *Richelia* symbionts were 171–420 times higher when the cells were symbiotic compared with estimates for cells living freely. The cyanobacterial symbiont fixed more N than needed for its own growth and in the case of *Richelia*, up to 97.3% fixed N was transferred to the diatom.

22.6.3 Animals

22.6.3.1 Introduction

The account of *Gloeotrichia echinulata* in Sect. 22.5.4 indicates that a lot needs to be known for successful application of a modelling approach to provide quantitative predictions. Nevertheless Benedetti-Cecchi et al. (2005) showed for a marine mid-littoral assemblage how a basic understanding of the mechanisms of interaction of the main species plus some

knowledge of their natural history can lead to correct predictions of the effects of one species on another. Filamentous algae monopolized the substratum when limpets were absent, but, when present *Rivularia,* the red alga *Rissoella* and barnacles colonized. Barnacles and *Rissoella* jointly reduced the coverage of *Rivularia* and the local density of limpets and this eventually led to colonization by filamentous algae late in succession. There was thus an intermediate stage in the succession when *Rivularia* was successful. The interactions are probably similarly complex in most ecosystems, but the following are simplified accounts of the main types of interaction between Rivulariaceae and animals..

22.6.3.2 Destruction of Whole Filaments

Rivulariaceae are of course frequently influenced by grazing and this is described for *Gloeotrichia echinulata* in Sect. 22.5.4. Sometimes there may be little selection for or against a particular cyanobacterium, as in the study by Theivandirrajah and Jeyaseelan (1977) on grazing of *Calothrix* and *Anabaena* by mosquito larvae in a Sri Lankan pond. However, Edmondson (1938) reported that the rotifer *Lindia euchromatica* in the littoral zone of Linley Pond, Connecticut, targeted *Gloeotrichia.* As it lived in the "slime" of the cyanobacterium, where it fed on the filaments and deposited its eggs, it is unclear whether this was *G. echinulata* or a species forming larger colonies. In the case of floating *G. natans* colonies in the Botanic Garden near Chiangmai, Thailand, in October 2011, the colonies contained three large species of testate amoebae, at least one of which was frequent and grazing *Gloeotrichia* trichomes by gradually engulfing and digesting them from the ends (B.A.W., unpublished data). *Lindia euchromatica* was the only grazer feeding on *G. echinulata* in Green Lake, Washington (Roelofs and Oglesby 1970). Although sometimes very abundant, it was noted on relatively few occasions and only in late summer. Colony size (up to 2 mm) was thought to restrict grazing by copepods and cladocerans. However, mesocosm experiments with *Daphnia pulex, Holopedium gibberum, Ceriodaphnia quadrangula* and *Bosmina longirostris* showed that they all increased the proportion of damaged colonies (Fey et al. 2010). *Ceriodaphnia* damaged the greatest proportion, but only *Daphnia pulex* fed on *Gloeotrichia echinulata* reproduced. Nothing is known about the impact of grazers on bottom sediments.

G. echinulata was one of a number of filamentous cyanobacteria cultures grazed by the amoeba *Naegleria* isolated from Dianchi Lake, Kunming, Yunnan, China (Liu et al. 2006). Forms with aggregated filaments such as *Aphanizomenon* were not grazed, which suggests that the *Gloeotrichia* culture had lost its colonial structure. Unicellular Chroococcales were ingested, but subsequently excreted. Nematodes are sometimes frequent in old colonies of *Rivularia biasolettiana*, but their possible role in destroying colonies has not been studied. The possibility of putting grazing to practical use as food for the apple snail *Pomacea patula catemacensis* which is endemic in Lake Catemaco, Mexico, was investigated by Ruiz-Ramírez et al. (2005). The survival of the snail, which is an important fishing resource, is threatened and hence there is a need to culture it. *Calothrix* sp. grown in 40-L containers proved to be better than pelleted carp food.

22.6.3.3 Partial Destruction of Filaments

A number of studies are known where Rivulariaceae populations appear especially successful in resisting grazing pressure. Several reasons have been put forward to explain this.

Mats of *Calothrix* embedded among *Pleurocaps*a persist in the lower parts of the temperature gradient of hot springs of the western USA, where grazing by ostracods eliminates other cyanobacteria capable of overgrowing these species (Wickstrom and Castenholz 1985). In the case of Hunter's Hot Springs, Oregon, *Calothrix* and *Pleurocapsa* withstood a dense population of *Potamocypris* sp., which exerted heavy grazing pressure on *Oscillatoria terebriformis*, the cyanobacterium dominating immediately upstream in the temperature zone where the ostracod was unable to persist (Castenholz 1973). The hairs of the *Calothrix*, which protrude from the colonies, are, however, heavily grazed (Castenholz, personal communication 1978). An experimental study (Power et al. 1988) showed that the dominance of *Calothrix* in a stream in Ozark Mountains, Oklahoma, depended on the presence of grazing by fish such as minnows. When the grazers were removed, the *Calothrix* mats became overgrown by diatoms within 4–10 days, while re-exposure to grazers led to the mats developing again. The authors suggested that the ability to regenerate from basal parts of the trichome may contribute to their persistence under intense grazing. They also discussed possible ecosystem-level effects, such as whether the removal of loosely attached diatoms contributed to the water clarity of Ozark streams dominated by cyanobacteria felts. This was in spite of the fact that dominance by the N_2-fixer brought about by the grazing might enhance N loading in the stream, as shown by Wilkinson et al. (1985) for the grazing of abundant N_2-fixing cyanobacteria (including *Calothrix*) on coral reefs.

22.6.3.4 Avoiding the Grazers

Cattaneo (1983) interpreted the success of *Gloeotrichia pisum* as an epiphyte in Lake Memphremagog, Québec-Vermont, as resulting from its ability to resist grazing by snails, which had a large impact on other algae. When snails were excluded, but not oligochaetes and chironomids, the control epiphyte community was replaced by green algae, mainly *Mougeotia*. It was suggested that the very tough sheath of *Gloeotrichia* may protect it from grazing. A laboratory experimental study (Osa-Afiana and Alexander 1981) showed that established populations of *Calothrix* and *Tolypothrix* were not grazed by the ostracod *Cypris* sp., whereas *Aulosir*a and, to a lesser extent, *Anabaena* were. However, when the cyanobacterium and ostracod were inoculated together, the ostracod increased

in biomass just as much with *Calothrix* as with *Anabaena*. Presumably *Calothrix* and *Tolypo*thrix formed sheaths in older cultures, though the authors did not discuss whether this was a factor reducing the effects of grazing.

Rivularia colonies sometimes persist for very long periods, which can be as much as 10 years in the case of *R. haematites* (Pentecost and Whitton 2000). As the presence of calcite crystals does not appear to inhibit grazing, at least by molluscs, toxicity was suggested as a likely factor. Several studies have now reported studies indicating its probable importance for at least some Rivulariaceae. The need for colonies to survive for many months under P-limited conditions with only slow growth during that period (Sect. 22.5) would add to the value of any means of detering grazers. The toxicity of calcified cyanobacterial communities from Spanish streams to stream invertebrates was investigated by Aboal et al. (2000, 2002). *Rivularia* was abundant in these communities, though other cyanobacteria were also well represented. The latter study, which generated index values for routine monitoring assays based on macroinvertebrates and diatoms, showed a clear inverse relationship between the dominance of cyanobacteria and the values obtained for the indices. Evidence for microcystin production by *Rivularia* in populations from N-E. and S-E. Spain was reported by Aboal et al. (2005), Aboal and Puig (2009): MC-LR, MC-RR and MC-YR were predominant. Microcystin-LR was also reported for *Gloeotrichia echinulata* (Carey et al. 2007). Among 19 cyanobacteria isolated from the sediments of R. Nile and adjacent channels, all of which were toxic to *Artemia*, extracts from *Calothrix parietina* and *Phormidium tenue* caused neurotoxic symptoms to mice within 10 h; five other species showed hepatotoxicity (Mohamed et al. 2006).

A different group of compounds was investigated by Höckelmann et al. (2009). This was the range of sesquiterpenes formed by the axenic geosmin-producing cyanobacterium, *Calothrix* PCC 7507, together with a comparison of their abundance in old *versus* very old standing cultures. Among the quantitatively important products, eremophilone differed from the others in being mainly excreted into the medium, so bioassays were conducted on its toxicity to three invertebrates. Acute toxicity to *Chironomus riparius* (insect) occurred at 29 μM and to *Thamnocephalus platyurus* (crustacean) at 22 μM, whereas no toxic effects were observed on *Plectus cirratus* (nematode). Neither 6.11-epoxyisodaucane nor isodihydroagarofuran exhibited toxicity to any of these at concentrations up to 100 μM.

22.6.4 Bacteria and Fungi

Sequencing and phylogenetic analysis of 16S rRNA genes of bacteria attached to *Gloeotrichia echinulata* colonies in Lake Erken showed a diverse community different from that in the main waterbody and included not only cyanobacteria, but populations affiliated with Proteobacteria, Bacteriodetes, Acidobacteria, Fusobacteria, Firmicutes and Verrucomicrobia (Eiler et al. 2006). Fan (1956) reported that the hyphae of certain fungi parasitize the cells of *Calothrix* and the cells or trichomes usually die. Although cyanophage activity is important for many other bloom-forming cyanobacteria, no studies have been reported for *Gloeotrichia echinulata*.

22.7 Geological Record and Environmental Change

As it is increasingly becoming possible to relate genera and often also particular species of modern-day Rivulariaceae to particular types of environment, their presence in the fossil record is also becoming important in interpreting past environments. If the interpretation of molecular data by Domínguez-Escobar et al. (2011) is correct (see Sect. 22.3.1), then *Calothrix* and *Rivularia* originated about ~1,500 Ma (Mesoproterozoic) and *Gloeotrichia* about and 400–300 Ma. (Carboniferous) This means that the doubts of Golubic and Campbell (1981) about fossil Rivulariaceae (mostly concerning *Palaeorivularia*) may not be justified. *Palaeocalothrix*, described from the Precambrian by Zhao-Liang (1984a, b) has a basal heterocyst, large akinete, trichome tapered toward the apex and a sheath, all of which are features of *Gloeotrichia* and possibly a few species of *Calothrix*. Assuming structures had a similar physiological significance then as in modern *Calothrix*, then *Palaeocalothrix* lived in a well oxygenated environment with a marked variation in some nutrient, probably P.

There are convincing records of Rivulariaceae for most geological periods from the Pleiocene onwards. These are a few examples. Dragastan et al. (1996) compared presumed *Rivularia haematites* from the Pleistocene and modern material. Based on tube dimensions and morphology they appear to be essentially the same. A 20-m core of Middle Pleistocene travertine showed encrustations of *Phormidium*, *Dichothrix* and *Rivularia* (Casanova 1984). Various Pliocene and Pleistocene deposits appear to include oncoids (see below) or similar structures formed by cyanobacteria (Casanova 1982, 1984). *Celyphus rallus* is almost certainly an Early Cretaceous Rivulariaceae (Batten and Van Geel 1985). Among the microfossils associated with Late Cretaceous freshwater stromatolites in Sonora, Mexico, Beraldi-Campesi et al. (2004) listed *Calothrix estromatolitica*. The use of cyanobacteria as biomarkers of hydrological changes in the Late Quaternary sediments of the South Kerala Sedimentary Basin in India, was assessed by Limaye et al. (2010).

A study of three different types of modern *Rivularia* colony (see Sect. 22.5.3) led Obenlüneschloss and Schneider (1991) to several conclusions relevant to interpreting fossil material. Once the organic matter has decomposed, it is

impossible to make direct conclusions on the trichome and sheath morphology based on the calcification structures. As several different calcification mechanisms occurred in these colonies, it is doubtful whether different calcification structures in fossil analogues can be attributed to different calcification mechanisms.

The possibility that carbonaceous meteorites might also contain fossil cyanobacteria has been presented in a number of papers by Hoover, with the latest (2011) arguing the case in detail. It includes the suggestion that several tapering structures with a smooth basal body in the Orgueil CI1 carbonaceous meteorite resemble the filaments with a heterocyst of a modern *Calothrix*, though the organism shown for comparison from White River, Washington, does not resemble typical *Calothrix*, including the fact that the filament is only 0.8 μm wide, apparently well below the limit for other records in the genus. The paper includes much of interest and led to rapid and vigorous internet discussion, some of it highly critical. It seems likely the debate will continue for some time before there is any scientific consensus about the possibility of these meteorites containing cyanobacteria, let alone that some of these resemble Rivulariaceae.

The presence of Rivulariaceae in relatively recent calcareous deposits has been reported by many authors based on both molecular (Sigler et al. 2003) and morphological data. *Calothrix* was among the cyanobacteria of stromatolitic stalagmites at the entrance to a cave in Slovenia (Mulec et al. 2007). The calcified remains of *Rivularia* colonies, especially *R. haematites*, often persist in or around streams and lake margins when they become exposed. Spherical or subspherical oncoids in small calcareous streams usually consist predominantly of *Rivularia* (Rott 1991) or almost entirely so, such as those at Sunbiggin, Cumbria, UK (illustrated in Whitton and Potts 2000). Based on the living algae found in Little Conestoga Creek, Roddy (1915) concluded that the calcareous structures all the way down the stream were formed largely by *Rivularia*, and possibly also the calcareous structures forming deep deposits under soil in the catchment. Oncoids had disappeared from this stream by the time it was resurveyed by Golubic and Fischer (1975), mostly likely because of acidic industrial effluents. Most records of the decrease (Kann 1982) or loss of calcifying *Rivularia* are, however, probably due largely to P enrichment (Pentecost and Whitton 2000). It also seems possible that atmospheric N deposition leading to increases in stream water N might shift away from N_2-fixers to eukaryotic algae adapted to environments with high N:P (Mateo et al. 2010).

The Rivulariaceae are usually frequent together with narrow sheathed Oscillatoriaceae in calcareous deposits on submerged rocks in shallow, highly calcareous lakes, where the deposits are sufficiently thick and well-laminated to justify the term stromatolite. The calcified layers forming stromatolites in Lough Corrib, Ireland, provide striking examples, with frequent *Dichothrix* being intermingled with abundant *Schizothrix fasciculata* (Fig. 22.10): see also article by B. Kennedy et al. (2012) in online supplement to this book. In larger calcareous lakes, such as the Bodensee (Lake Constance), benthic rocks can have a thick calcareous layer with a furrow or brain-like pattern (Müller-Stoll 1986), which is formed mainly by *Schizothrix fasciculata* and *Rivularia haematites* in the Bodensee; the author lists reports of other lakes with similar structures. Oncoids form where detached calcareous growths are moved around by weak currents, such as the floor of the Untersee of Lake Constance, where they consist of *Phormidiu*m and *Calothrix* and/or *Dichothrix* (Schäfer and Stapf 1978). A study (Van Geel et al. 1984) of Lateglacial deposits near Usselo, The Netherlands, gives detailed records of changes in "*Gloeotrichia*-type", though the authors did not give details of the structures counted. The sequence, which started in an oligotrophic shallow pool with very low organic production in a barren sandy-landscape, had an early phase at about 12,600 B.P., but lasting several hundred years, characterized as the *Gloeotrichia*-type, where Characeae were also important. The authors suggested N_2 fixation may have initiated nutrient availability.

As mentioned in Sect. 22.5.3, Rivulariaceae sometimes calcify in the marine environment. This was reported for *Dichothrix* at Highbourne Cay, Bahamas (Planavsky et al. 2009), but the filaments were not preserved to form lithified microbialites. In contrast, adjacent cyanobacterial mats formed well-laminated stromatolites. However, *Dichothrix* contributed to one of the types of thrombolite described by Mobberley et al. (2011) and carbonate-trapping stromatolites in the marine environment can be formed by *D. bornetiana* (Monty 1967).

22.8 Discussion

Although molecular data show that the Rivulariaceae are a heterogeneous group, field and experimental observations indicate that they are all well adapted to environments with marked variation in ambient P, often organic P in particular. In flowing waters and the marine intertidal this variation is temporal, but it is both temporal and spatial in *Gloeotrichia echinulata*. The periodicity of temporal variation in *Calothrix* is much shorter than in colonial forms and in *Dichothrix* probably shorter and more irregular than in most *Rivularia*. When ambient P concentrations drop, stored polyphosphate is metabolized and surface phosphatase activities increase, permitting efficient use of organic phosphates in the environment. Hormogonia formation ceases when internal P concentrations fall below a particular level. Taxa differ considerably in the relative extent of their P-rich and P-limited periods. *G. echinulata* in the eutrophic Lake Erken is probably P-rich for much of the time it is growing actively, but there

may be longer periods in other lakes when the species is at least moderately P-limited.

Rivularia is at the other extreme and in temperate region streams it is P-limited for much of the year. In this case the long periods of presumably low metabolic activity and the persistence of colonies for many years at some sites makes it important for grazing activity to be minimized; their formation of microcystins needs to be studied in detail. The presence of more than one genotype in the colonies of at least some *Rivularia* populations may provide flexibility in responding to longer term fluctuations in the environment at the site. Presumably the genotype best suited to the current environment grows the most rapidly, but perhaps the relative proportions of different genotypes are also controlled at the stage when trichomes first aggregate to form colonies.

The ability of some species to form long colourless hairs greatly increases the surface for phosphatase activities and probably also uptake of inorganic P. In colonial forms hairs also help to enhance the size of the colony. However, the production of hairs leads to the loss of cells each time the trichome becomes P-rich. There is no such wastage in species which do not form hairs. Hairs permit the trichome to use very low ambient P concentrations, yet also make efficient use of pulses of higher P concentration. It also seems possible that hair cell formation in some species leads to changes in the cytoplasmic membrane that permit passage of organic phosphates, which are then hydrolyzed inside the cell. The need to reduce entry of seawater might explain why *Calothrix viguieri* only forms hairs in freshwater (Sect. 22.4.2). Nevertheless, most marine Rivulariaceae do possess hairs. Once phosphate ions are released in the hair, rapid transfer must occur to the base near of the trichome where polyphosphate granule formation is first evident.

Although this review has emphasized the importance of P in determining the growth cycle, the possibility that Fe may have a similar influence in some ecological situations should be borne in mind. The cycle of hair and hormogonia formation in response to Fe status reported for *Calothrix* strains in Sects. 22.2.2.1 and 22.2.2.3 is probably an indirect response to P limitation. Nevertheless, there may be environments where marked changes in Fe availability are the key factor and perhaps there were past geological periods when it was especially important. Apart from this uncertainty, understanding of how morphology relates to the environment is sufficiently advanced that it should be possible to characterize the environment of all distinctive taxa of Rivulariaceae. However, the fact that colonies may include more than one genotype indicates the likelihood of a continuum of forms in many types of environment. Molecular information about how different taxa respond to changes in internal P status would be a great help, as would establishing whether or not morphological changes are sometimes a response to external P concentration or types of molecule. Nevertheless sufficient is known about the ecology of Rivulariaceae to comment on the environment of fossil material and old lake plankton records in some detail.

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References

Abe T, Ikeda T, Yanada R, Ishikura M (2011) Concise total synthesis of calothrixins A and B. Org Lett 13(13):3356–3359

Aboal M, Puig MA (2009) Microcystin production in *Rivularia* colonies of calcareous streams from Mediterranean Spanish basins. Algol Stud 130:39–52

Aboal M, Puig MA, Rios H, López-Jiménez E (2000) Relationship between macroinvertebrate diversity and toxicity of cyanophyceae (Cyanobacteria) in some streams from eastern Spain. Verh Int Verein Limnol 27:555–559

Aboal M, Puig MA, Mateo P, Perona E (2002) Implications of cyanophyte toxicity on biological monitoring of calcareous streams in north-east Spain. J Appl Phycol 14:49–56

Aboal M, Puig MA, Asencio AD (2005) Production of microcystins in calcareous Mediterranean streams: the Alharabe River, Segura River basin in south-east Spain. J Appl Phycol 17:231–243

Adamec F, Kaftan D, Nedbal L (2005) Stress-induced filament fragmentation of *Calothrix elenkinii* (Cyanobacteria) is facilitated by death of high-fluorescence cells. J Phycol 41:835–839

Adams DG (2000) Symbiotic interactions. In: Whitton B, Potts M (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 523–561, 668 pp

Ahmadjian V (1967) A guide to the algae occurring in lichen symbionts: isolation, culture, cultural physiology, and identification. Phycologia 6:127–160

Al-Mousawi AHA (1984) Biological studies on algae in ricefield soil from the Iraqi marshes. Ph.D. thesis, University of Durham, Durham

Al-Mousawi AHA, Whitton BA (1983) Influence of environmental factors on algae in rice-field soil from the Iraqi marshes. Arab J Gulf Res 1:237–253

Aziz A (1985) Blue-green algal nitrogen fixation associated with deepwater rice in Bangladesh. Ph D thesis, University of Durham, Durham

Aziz A, Whitton BA (1988) Influence of light flux on nitrogenase activity of the deepwater rice-field cyanobacterium (blue-green alga) *Gloeotrichia pisum* in field and laboratory. Microbios 53:7–19

Aziz A, Whitton BA (1989) Morphogenesis of blue-green algae. II. Hair differentiation in *Gloeotrichia pisum*. Bangladesh J Bot 16:69–81

Banerjee M, John J (2005) Phosphatase activity of non-hair forming cyanobacterium *Rivularia* and its role in phosphorus dynamics in deepwater rice-fields. Appl Ecol Environ Res 3(1):55–60

Barbiero RP (1993) A contribution to the life history of the planktonic cyanophyte, *Gloeotrichia echinulata*. Arch Hydrobiol 127:87–100

Barbiero RP, Welch EB (1992) Contribution of benthic blue-green algal recruitment to lake populations and phosphorus recruitment. Freshw Biol 27:249–260

Bary D (1863) Beitrag zur Kenntnis der Nostocaceen, insbesondere Rivulariaceen. Flora (Jena) 46:553–560

Batten DL, Van Geel B (1985) *Celyphus rallu*s, probably early Cretaceous rivulariacean blue-green alga. Rev Palaeobot Palynol 44:233–241

Bauersachs T, Compaoré J, Hopmans EC, Stal LJ, Schouten S, Damsté JSS (2009) Distribution of heterocyst glycolipids in cyanobacteria. Phytochemistry 70:2034–2039

Benedetti-Cecchi L, Vaselli S, Maggi E, Bertocci I (2005) Interactive effects of spatial variance and mean intensity of grazing on algal cover in rock pools. Ecology 86:2212–2222

Beraldi-Campesi H, Cevallos-Ferriz SRS, Chacón-Baca E (2004) Microfossil algae associated with Cretaceous stromatolites in the Tarahumara Formation, Sonora, Mexico. Cretac Res 25:249–265

Bernardo PH, Chai CLL, Heath GA, Mahon PJ, Smith GD, Waring P, Wilkes BA (2004) Synthesis, electrochemistry, and bioactivity of the cyanobacterial calothrixins and related quinones. J Med Chem 47:4958–4963

Bernardo PH, Chai CLL, Le Guen M, Smith GD, Waring P (2007) Structure-activity delineation of quinones related to the biologically active calothrixin B. Bioorg Med Chem Lett 17(1):82–85

Berrendero E, Perona E, Mateo P (2008) Genetic and morphological characterization of *Rivularia* and *Calothrix* (Nostocales, Cyanobacteria) from running water. Int J Syst Evol Microbiol 58:447–460

Berrendero E, Perona E, Mateo P (2011) Phenotypic variability and phylogenetic relationships of *Tolypothrix* and *Calothrix* (Nostocales, Cyanobacteria) from running water. Int J Syst Evol Microbiol 61(12):3039–3051

Böcher TW (1946) *Dichothrix gelatinosa* sp. n., its structure and resting organs. K Danske Vidensk Selsk Skr IV(4):1–15

Bornet JBE, Flahault CGM (1886) Revision des Nostocacées hétérocystées contenues dans les principaux herbiers de France. Ann Sci Nat Bot Sér 7(3):323–381

Campbell D, Houmard J, Tandeau de Marsac N (1993) Electron transport regulates cellular differentiation in the filamentous cyanobacterium *Calothrix*. Plant Cell 5(4):451–463

Campos IFP, Senna PAC (1988) Nostocophyceae (Cyanophyceae) de Lagao Bonita, Distrito Federal, Brasil. Parto 1. Acta Bot Bras 2(1–2):7–30

Capone DG, Taylor BF (1977) Nitrogen fixation (acetylene reduction) in the phyllosphere of *Thalassia testidinum*. Mar Biol 40(1): 19–28

Carey CC, Rengefors K (2010) The cyanobacterium *Gloeotrichia echinulata* stimulates the growth of other phytoplankton. J Plankton Res 32:1349–1354

Carey CC, Haney JF, Cottingham KL (2007) First report of microcystin-LR in the cyanobacterium *Gloeotrichia echinulata*. Environ Toxicol 22:337–339

Carey CC, Weathers KC, Cottingham KL (2008) *Gloeotrichia echinulata* blooms in an oligotrophic lake helpful insights from eutrophic lakes. J Plankton Res 30:893–904

Carey CC, Weathers KC, Cottingham KL (2009) Increases in phosphorus at the sediment-water interface may influence the initiation of cyanobacterial blooms in an oligotrophic lake. Verh Int Verein Limnol 30(8):1185–1188

Carey CC, Cottingham KL, Weathers KC, Brentrup JA, Ruppertsberger NM, Ewing HA, Hairston NG (2011) Cyanobacteria are not all bad: *Gloeotrichia echinulata* may stimulate plankton food webs in nutrient-limited freshwater ecosystems. In: Abstract, 96th Ecological Society of America annual meeting, Austin, TX, 7–12 Aug 2011

Carpenter EJ, Cox JL (1974) Production of *pelagic* Sargassum and a blue-green epiphyte in the western Sargasso Sea. Limnol Oceanogr 19:429–436

Casanova J (1982) Morphologie et biolithogenèse des barrages de travertins. In: Nicod J (ed) Formations Carbonatées -Externes, Tufs et Travertins. Institut de géographie, Aix-en-Provence, pp 45–54

Casanova J (1984) Genèse des carbonates d'un travertin pléistocène: interpretation paleoecologique du sondage Peyr I (Comprégnac, Aveyron). Geobios 17:219–229

Castenholz RW (1973) Ecology of the blue-green algae in hot springs. In: Carr NG, Whitton BA (eds) The biology of the blue-green algae. Blackwell, Oxford, pp 379–414, 655 pp

Castenholz RW (1989) Rivulariaceae. In: Bergey's manual of systematic bacteriology 3. Williams & Wilkins, Baltimore, pp 1790–1793

Cattaneo A (1983) Grazing on epiphytes. Limnol Oceanogr 28:124–132

Caudwell C, Lang J, Pascal A (1997) Étude expérimentale de la lamination stromatolithes à *Rivularia haematites* mat en climat tempéré: édification des lamines microcritiques. C R Acad Sci Paris 324(IIa): 883–890

Caudwell C, Lang J, Pascal A (2001) Lamination of swampy-rivulets *Rivularia haematites* stromatolites in a temperate climate. Sediment Geol 143:125–147 (+ 148: 451)

Chang TP (1979a) Growth and acetylene reduction by *Gloeotrichia echinulata* (Smith) Richter in axenic culture. Br Phycol J 14:207–210

Chang TP (1979b) Zur Morphologie von *Gloeotrichia echinulata* (Smith) Richter in axenischer Kultur. Nova Hedwigia Kryptogamenk 21:265–283

Chang T-P (1983) Interaction of water-blooming cyanophyte *Gloeotrichia echinulata* and its endophytic blue-green alga *Pseudanabaena catenata*. Arch Hydrobiol 97:320–328

Chang T-P, Blauw TS (1980) Nitrogen fixing capacity of two colonial types of *Gloeotrichia echinulata* and its endophytic blue-green alga *Pseudanabaena catenata*. Arch Hydrobiol 89:382–386

Chen X, Smith GD, Waring P (2003) Human cancer cell (Jurkat) killing by the cyanobacterial metabolite calothrixin A. J Appl Phycol 15:269–277

Claassen MI (1973) Freshwater algae of Southern Africa. I. Notes on *Gloeotrichia ghosei* R.N.Singh. Br Phycol J 8:325–331

Cuzman OA, Stefano Ventura S, Sili C, Mascalchi C, Tulio Turchetti T, D'Acqui LP, Tiano P (2010) Biodiversity of phototrophic biofilms dwelling on monumental fountains. Microb Ecol 60:81–95

Darley J (1967) Sur quelques résultats de la culture en laboratoire de deux espèces de *Calothrix* Agardh (Myxophycées-Rivulariacées). C R Acad Sci Ser 264D:1013–1015

de Toni G (1936) Noterelle di Nomencl. Algological 8:5

Desikachary TV (1946) Germination of the heterocyst in two members of the Rivulariaceae, *Gloeotrichia raciboskii* Wolosz. and *Rivularia mangini* Frémy. J Indian Bot Soc 25:11–17

Desikachary TV (1959) Cyanophyta. Indian Council of Agricultural Research, New Delhi, 686 pp

Dillon JG, Castenholz RW (2003) The synthesis of the UV-screening pigment, scytonemin, and photosynthetic performance in isolates from closely related natural populations of cyanobacteria (*Calothrix* sp.). Environ Microbiol 5(6):484–491

Dillon JG, Miller SR, Castenholz RW (2003) UV-acclimation responses in natural populations of cyanobacteria (*Calothrix* sp.). Environ Microbiol 5:473–483

Doan NT, Rickards RW, Rothschild JM, Smith GD (2000) Allelopathic actions of the alkaloid 12-*epi*-hapalindole E isonitrile and calothrixin A from cyanobacteria of the genera *Fischerella* and *Calothrix*. J Appl Phycol 12:409–416

Doan NT, Stewart PR, Smith GD (2001) Inhibition of bacterial RNA polymerase by the cyanobacterial metabolites 12-*epi*-hapalindole E isonitrile and calothrixin A. FEMS Microbiol Lett 196:135–139

Domínguez-Escobar J, Beltrán Y, Bergman B, Díez B, Ininbergs K, Souza V, Falcón LI (2011) Phylogenetic and molecular clock inferences of cyanobacterial strains within Rivulariaceae from distant environments. FEMS Microbiol Lett 316:90–99

Donaldson A, Whitton BA (1977) Algal flora of freshwater habitats on Aldabra. Atoll Res Bull 215:1–26

Douglas D, Peat A, Whitton BA, Wood P (1986) Influence of iron status on structure of the cyanobacterium (blue-green alga) *Calothrix parietina*. Cytobios 47:155–165

Dragastan G, Golubic S, Richter DK (1996) *Rivularia haematite*s: a case study of the recent versus fossil morphological. Taxonomical considerations. Rev Española Micropaleontol 28:43–73

Dromgoole FI, Silvester WB, Hicks BJ (1978) Nitrogenase associated with *Codium* species from New Zealand. N Z J Mar Freshw Res 12:17–22

Duong TP, Tiedje JM (1985) Nitrogen fixation by naturally occurring duckweed-cyanobacterial associations. Can J Microbiol 31: 327–330

Edmondson WT (1938) Three new species of Rotatoria. Trans Am Microsc Soc 57:153–157

Ehrenreich IM, Waterbury JB, Webb EA (2005) Distribution and diversity of natural product genes in marine and freshwater cyanobacterial cultures and genomes. Appl Environ Microbiol 71(11):7401–7413

Eiler A, Olsson JA, Bertilsson S (2006) Diurnal variations in the auto- and heterotrophic activity of cyanobacterial phycospheres (*Gloeotrichia echinulata*) and the identify of attached bacteria. Freshw Biol 51:298–311

Falcón LI, Magall Falcón S, Castillo A (2010) Dating the cyanobacterial ancestor of the chloroplast. ISME J 4:777–783

Fan KC (1956) Revision of *Calothrix* Ag. Rev Algol N.S. 2(3): 154–178

Fey SB, Mayer ZA, Davis SC, Cottingham KL (2010) Zooplankton grazing of *Gloeotrichia echinulata* and associated life history consequences. J Plankton Res 9:1337–1347

Fogg GE (1969) The physiology of an algal nuisance. Proc R Soc Lond B 173:175–189

Forsell L, Pettersson K (1995) On the seasonal migration of the cyanobacterium *Gloeotrichia echinulata* in Lake Erken, Sweden, and is influence on the pelagic population. Mar Freshw Res 46:287–293

Foster RA, Zehr JP (2006) Characterization of diatom-cyanobacteria symbioses on the basis of *nif*H, *het*R and 16S rRNA sequences. Environ Microbiol 8:1913–1925

Foster RA, Capone DG, Carpenter EJ, Mahaffey C, Subramaniam A, Zehr JP (2007) Influence of the Amazon River Plume on distributions of free-living and symbiotic cyanobacteria in the Western Tropical North Atlantic Ocean. Limnol Oceanogr 52:517–532

Foster RA, Subramaniam A, Zehr JP (2009) Distribution and activity of diazotrophs in the Eastern Equatorial Atlantic. Environ Microbiol 11:741–750

Foster RA, Goebel NL, Zehr JP (2010) Isolation of *Calothrix rhizosoleniae* (cyanobacteria) strain sc01 from *Chaetoceros* (Bacillariophyta) spp. diatoms of the subtropical North Pacific Ocean. J Phycol 46:128–1037

Foster RA, Kuypers MMM, Vagner T, Paerl RW, Muzat N, Zehr JP (2011) Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses. ISME J 5:1484–1493. doi:10.1038/ismej.2011.26

Friedmann I (1956) Über der Blaualga *Gardnerula corymbosa* (Harvey) J. De Toni und ihr Vorkommen im Mittelmeer. Öst Bot Z 103:336–341

Fritsch FE (1907a) The subaerial and freshwater algal flora of the Tropics. Ann Bot 21:235–275

Fritsch FE (1907b) A general consideration of the freshwater algae and subaerial algae of Ceylon. Proc R Soc Lond B 79:197–254

Geitler L (1932) Cyanophyceae. In: Rabenhorst's Kryptogamen-flora, vol 14. Akademische Verlagsgesellschaft, Leipzig, 1196 pp

Golden SS (1995) Light-responsive gene expression in cyanobacteria. J Bacteriol 177:1651–1654

Golubic S, Campbell SE (1981) Biogenically formed aragonite concretions in marine *Rivulari*a. In: Monty C (ed) Phanerozoic stromatolites. Springer, Berlin/Heidelberg, pp 209–229

Golubic S, Fischer AG (1975) Ecology of calcareous nodules forming in Little Connestoga Creek, near Lancaster, Pennsylvania. Verh Int Verein Limnol 19:2315–2323

Gomont M(A) (1895) Note sur un Calothrix sporifère (Calothrix stagnalis sp. n.). J Bot 9:1–6

Gonsalves EA, Kamat ND (1960) New species of Cyanophyceae from Mysore State – 1. J Bombay Nat Hist Soc 57(2):454–456

Grainger SLJ, Peat A, Tiwari DN, Whitton BA (1989) Phosphomonoesterase activity of the cyanobacterium (blue-green alga) *Calothrix parietina*. Microbios 59:7–17

Gunasekera SP, Miller MW, Kwan JC, Luesch H, Paul VJ (2010) Molassamide, a depsipeptide serine protease inhibitor from the marine cyanobacterium *Dichothrix utahensis*. J Nat Prod 73:459–462

Healey FP (1982) Phosphate. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publishers, Oxford, pp 105–124, 688 pp

Henssen A (1969) Three non-marine species of the genus *Lichina*. Lichenologist 4:88–98

Henssen A (1973) New or interesting cyanophytic lichens. I. Lichenologist 5:444–451

Heuff H, Horkan K (1984) Caragh. In: Whitton BA (ed) Ecology of European rivers. Blackwell, Oxford, pp 363–384, 644 pp

Hicks BJ, Silvester WB (1985) Nitrogen fixation associated with the New Zealand mangrove (*Avicennia marina* (Forsk.) Vierh. var. *resinifera* (Forst. f.) Bakh.). Appl Environ Microbiol 49:955–959

Höckelmann C, Becher PG, von Reuss SH, Jüttner F (2009) Sesquiterpenes of the geosmin-producing cyanobacterium *Calothrix* PCC 7507 and their toxicity to invertebrates. Z Naturforsh 64C:49–55

Hongmei J, Aitchison JC, Lacap DC, Peerapornpisal Y, Sompong U, Pointing SB (2005) Community phylogenetic analysis of moderately thermophilic cyanobacterial mats from China, the Philippines and Thailand. Extremophiles 9:325–332

Hoover RB (2011) Fossils of cyanobacteria in CI1 carbonaceous meteorites. J Cosmol 13:1–39

Huang TC, Grobbelaar N (1989) Isolation and characterization of endosymbiotic *Calothrix.* (Cyanophyceae) in *Encephalartos hildebrandtii* (Cycadales). Phycologia 28:464–468

Hübel H, Hübel M (1974) In-situ Messungen der diurnalen Stickstoff Fixierung an Mikrobenthos der OstseeKüste. Arch Hydrobiol Suppl 46:39–54

Hyenstrand P, Rydin E, Gunnerhed M, Linder J, Blomqvist P (2001) Response of the cyanobacterium *Gloeotrichia echinulata* to iron and boron additions – an experiment from Lake Erken. Freshw Biol 46:735–741

Islam MR, Whitton BA (1992a) Phosphorus content and phosphatase activity of the deepwater rice-field cyanobacterium (blue-green alga) *Calothrix* D764. Microbios 69:7–16

Islam MR, Whitton BA (1992b) Cell composition and nitrogen fixation by the deepwater rice -field cyanobacterium (blue-green alga) *Calothrix* D764. Microbios 69:77–88

Istvánovics V (2008) The role of biota in shaping the phosphorus cycle in lakes. Freshw Rev 1:143–174

Istvánovics V, Pettersson K, Pierson D (1990) Partitioning of phosphate uptake between different size groups of planktonic microorganisms in Lake Erken. Verh Int Verein Limnol 24:231–245

Istvánovics V, Pettersson K, Rodrigo MA, Pierson D, Padisák J, Colom W (1993) *Gloeotrichia echinulata*, a colonial cyanobacterium with a unique phosphorus uptake and life strategy. J Plankton Res 15:531–552

Janson S, Rai AN, Bergman B (1993) The marine lichen *Lichina confinis* (O. F. Müll.) C. Ag.: ultrastructure and localization of nitrogenase, glutamine synthetase, phycoerythrin and ribulose 1, 5-bisphosphate carboxylase/oxygenase in the cyanobiont. New Phytol 124:149–160

Johansson C (1979) Chlorophyll content and the periphytic algal vegetation in six streams in North Jämtland, Sweden, 1977. Medd Växtbiol Inst Uppsala 1979(2):1–28

Jones K, Stewart WDP (1969) Nitrogen turnover in marine and brackish habitats III. The production of extracellular nitrogen by *Calothrix scopulorum*. J Mar Biol Assoc UK 49:475–488

Kann E (1982) Qualitative Veränderungen der litoralen Algenbiocönose österreichischer Seen. Arch Hydrobiol Suppl 62:440–490

Karlsson I (2003) Benthic growth of *Gloeotrichia echinulata* cyanobacteria. Hydrobiologia 506:189–193

Karlsson-Elfgren I, Rydin E, Hyenstrand P, Pettersson K (2003) Recruitment and pelagic growth of *Gloeotrichia echinulata* in Lake Erken. J Phycol 39:1050–1056

Karlsson-Elfgren I, Rengefors K, Gustafson S (2004) Factors regulating recruitment from the sediment to the water column in the bloom-forming cyanobacterium *Gloeotrichia echinulata*. Freshw Biol 49:265–273

Karthikeyan N, Prasanna R, Sood A, Jaiswal P, Nayak S, Kaushik BD (2009) Physiological characterization and electron microscopic investigation of cyanobacteria associated with wheat rhizosphere. Folia Microbiol 54:43–51

Katayama M, Kobayashi M, Ikeuchi M (2007) Phototropism observed in cyanobacterium *Rivularia* sp. Plant Cell Physiol 48:158

Kenyon CN, Rippka R, Stanier RY (1972) Fatty acid composition and physiological properties of some filamentous blue-green algae. Arch Mikrobiol 83:216–236

Khan QA, Lu J, Hecht SM (2009) Calothrixins, a new class of human DNA topoisomerase I poisons. J Nat Prod 72(3):438–442

Khoja TM, Whitton BA (1971) Heterotrophic growth of blue-green algae. Arch Mikrobiol 79:280–282

Khoja TM, Livingstone D, Whitton BA (1984) Ecology of a marine *Rivularia* population. Hydrobiologia 108:65–73

Kirkby SM, Whitton BA (1976) Uses of coded data in study of *Calothrix* and *Rivularia*. Br Phycol J 11:407–416

Lachance M-A (1981) Genetic relatedness of heterocystous cyanobacteria by deoxyribonucleic acid-deoxyribonucleic acid reassociation. Int J Syst Bacteriol 31:13–147

Lami R, Meslin R (1959) Sur une cyanophycée, *Calothrix Chapmanii* nom. nov., vivant à l'intérieur d'une Entéromorphe limicole. Bull Lab Marit Dinard 44:47–49

Lange W (1974) Chelating agents and blue-green algae. Can J Microbiol 20(10):1311–1320

Larsen LK, Re M, Patterson GML (1994) β-carbolines from the blue-green alga *Dichothrix baueriana*. J Nat Products 57(3): 419–421

Lebedeva NV, Boichenko VA, Semenova LR, Pronina NA, Stadnichuk IN (2005) Effects of glucose during photoheterotrophic growth of the cyanobacterium *Calothrix* sp PCC 7601 capable for chromatic adaptation. Russ J Plant Physiol 52:235–241

Lemmermann E (1905) Die Algenflora der Sandwich-Islen. Ergebnisse einer Reise nach dem Pacific. H. Schauinsland 1896/97. Bot Jahrbuch Systematik Pflanzengesch Pflanzengeogr 3:607–663

Limaye RB, Kumaran KPN, Nair KM, Padmalal D (2010) Cyanobacteria as potential biomarkers of hydrological changes in the Late Quaternary sediments of South Kerala Sedimentary Basin, India. Quaternary Int 213:79–90

Liu Xinyao, Shi Miao, Liao Yonghong, Gao Yin, Zhang Zhongkai, Wen Donghui, Wu Weizhong (2006) An Chencai Feeding characteristics of an amoeba (Lobosea: *Naegleria*) grazing upon cyanobacteria: food selection, ingestion and digestion progress. Microb Ecol 31:315–325

Livingstone D, Whitton BA (1983) Influence of phosphorus on morphology of *Calothrix parietina* (Cyanophyta) in culture. Br Phycol J 18:29–38

Livingstone D, Whitton BA (1984) Water chemistry and phosphatase activity of the blue-green alga *Rivularia* in Upper Teesdale streams. J Ecol 72:405–421

Livingstone D, Khoja TM, Whitton BA (1983) Influence of phosphorus on physiology of a hair-forming blue-green alga (*Calothrix parietina*) from an upland stream. Phycologia 22:345–350

Livingstone D, Pentecost A, Whitton BA (1984) Diel variations in nitrogen and carbon dioxide fixation by the blue-green alga *Rivularia* in an upland stream. Phycologia 23:125–133

Maehr H, Smallheer JM (1984) Rivularins. Preliminary synthetic studies. Org Chem 49:1549–1553

Mahasneh IA, Grainger SLJ, Whitton BA (1990) Influence of salinity on hair formation and phosphatase activities of the blue-green alga (cyanobacterium) *Calothrix viguieri* D253. Br Phycol J 25: 25–329

Mateo P, Douterelo I, Berrendero E, Perona E (2006) Physiological differences between two species of cyanobacteria in relation to phosphorus limitation. J Phycol 42:61–66

Mateo P, Berrendero E, Perona E, Loza V, Whitton BA (2010) Phosphatase activities of cyanobacteria as indicators of nutrient status in a Pyrenees river. Hydrobiologia 652:255–268

Maxwell TF (1974) Developmental morphology of the blue-green alga, *Gloeotrichia echinulata*. J Phycol 10 (Suppl):4

McErlean CSP, Sperry J, Blake AJ, Moody CJ (2007) Synthesis of the calothrixins, pentacyclic indolo[3,2-*j*]phenanthridine alkaloids, using a biomimetic approach. Tetrahedron 63(45):10963–10970

Mobberley JM, Ortega MC, Foster JS (2011) Comparative microbial diversity analyses of modern marine thrombolitic mats by barcoded pyrosequencing. Environ Microbiol. doi:10.1111/j.1462-2920.2011.02509.x

Mohamed ZA, El-Sharouny HM, Ali WSM (2006) Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigation canals, Egypt. Toxicon 47:584–590

Monty CLV (1967) Distribution and structure of recent stromatolitic algal mats, eastern Andros Island, Bahamas. Ann Soc Geol Belg Bull 96:585–624

Mulec J, Kosi G, Vrhovsek D (2007) Algae promote growth of stalagmites and stalactites in karst caves (Skocjanske jame, Slovenia). Carbonates Evaporites 22:6–9

Müller-Stoll WR (1986) Der Cyanophyceen-Bewuchs der Furchen- oder Hirnsteine des Bodensees. Carolinea 44:51–60

Myshrall KL, Mobberley JM, Green SJ, Visscher PT, Havemann SA, Reid RP, Foster JS (2010) Biogeochemical cycling and microbial diversity in the thrombolitic microbialites of Highborne Cay, Bahamas. Geobiology 8(4):337–354

Norton RS, Wells RJ (1982) A series of chiral polybrominated biindoles from the blue-green marine algae *Rivularia firma*. Applications of ^{13}C NMR spin-lattice relaxation data and 123H-1H coupling constants to structure elucidation. J Am Chem Soc 104:3628–3635

Obenlüneschloss J, Schneider J (1991) Ecology and calcification patterns of *Rivularia* (Cyanobacteria). Algol Stud 64:489–502

Osa-Afiana LO, Alexander M (1981) Factors affecting predation by a microcrustacean (*Cypris* sp.) on nitrogen-fixing blue-green algae. Soil Biol Biochem 13:27–32

Pedersen M, DaSilva EJ (1973) Simple brominated phenols in the blue-green alga *Calothrix brevissima* West. Planta 115:83–86

Pentecost A (1987) Growth and calcification of the freshwater cyanobacterium *Rivularia haematites*. Proc R Soc Lond B 232:125–136

Pentecost A, Franke U (2010) Photosynthesis and calcification of the stromatolitic freshwater cyanobacterium *Rivularia*. Eur J Phycol 45:345–353

Pentecost A, Riding R (1986) Calcification in cyanobacteria. In: Leadbeater BSC, Riding R (eds) Biomineralization of lower plants and animals. Clarendon, Oxford, pp 73–90

Pentecost A, Whitton BA (2000) Limestones. In: Whitton BA, Potts M (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 257–279, 668 pp

Pereira I, Moya M, Reyes G, Kramm V (2005) A survey of heterocystous cyanobacteria in Chilean rice-fields. Gayana Bot 62(1):26–32

Pettersson K (1980) Alkaline phosphatase activity and algal surplus phosphorus as phosphorus deficiency indicators in Lake Erken. Arch Hydrobiol 89:54–87

Pettersson K, Istvánovics V, Pierson D (1990) Effects of vertical mixing on phytoplankton phosphorus supply during summer in Lake Erken. Verh Int Verein Limnol 24:236–241

Pettersson K, Herlitz E, Istvánovics V (1993) The role of *Gloeotrichia echinulata* in the transfer of phosphorus from sediments to water in Lake Erken. Hydrobiologia 253:123–129

Phillips W (1884) The breaking of the Shropshire meres. Trans Shropshire Archaeol Nat Hist Soc 7:277–300

Planavsky N, Reid RP, Lyons TW, Myshrall KL, Visscher PT (2009) Formation and diagenesis of modern marine calcified cyanobacteria. Geobiology 7:566–576

Potts M, Whitton BA (1977) Nitrogen fixation by blue-green algal communities in the intertidal zone of the Lagoon of Aldabra Atoll. Oecologia 27:275–283

Power ME, Stewart AJ, Matthews WJ (1988) Grazer control of algae in an Ozark mountain stream: effects of short-term exclusion. Ecology 69:1894–1898

Prasanna R, Pabby A, Saxena S, Singh PK (2004a) Modulation of pigment profiles of *Calothrix elenkenii* in response to environmental changes. J Plant Physiol 161:1125–1132

Prasanna R, Pabby A, Singh PK (2004b) Effect of glucose and light-dark environment on pigmentation profiles in the cyanobacterium *Calothrix elenkenii*. Folia Microbiol (Praha) 49:26–30

Querijero-Palacpac NM, Martinez MR, Boussiba S (1990) Mass cultivation of the nitrogen-fixing cyanobacterium *Gloeotrichia natans,* indigenous to rice-fields. J Appl Phycol 2:319–325

Rai AK, Pandey KD, Kashyap AK (1978) Heterocyst differentiation in *Calothrix*. New Phytol 81:647–651

Reed RH, Stewart WDP (1983) Physiological responses of *Rivularia atra*: osmotic adjustment to hypersaline media. New Phytol 95:595–603

Reyes J, Sansón M (1997) Temporal distribution and reproductive phenology of the epiphytes on *Cymodocea nodosa* leaves in the Canary Islands. Bot Mar 40:1–6

Rickards RW, Rothschild JM, Willis AC, de Chazal NM, Kirk J, Kirk K, Saliba KJ, Smith GD (1999) Calothrixins A and B, novel pentacyclic metabolites from *Calothrix* cyanobacteria with potent activity against malaria parasites and human cancer cells. Tetrahedron 55(47): 13513–13520

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Rippka R, Castenholz RW, Herdman M (2001a) Subsection IV.II form-genus I. *Calothrix* Agardh 1824. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 582–585, 721 pp

Rippka R, Castenholz RW, Herdman M (2001b) Subsection IV.II form-genus II. *Rivularia* Agardh 1824. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 586–587, 721 pp

Rippka R, Castenholz RW, Herdman M (2001c) Subsection IV. (Formerly Nostocales Castenholz 1989b sensu Rippka, Deruelles, Waterbury, Herdman and Stanier 1979) In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology. vol 1, 2nd edn. Springer, New York, pp 562–599, 721 pp

Roddy HJ (1915) Concretions in streams formed by the agency of blue green algae and related plants. Proc Am Phil Soc 54:246–258

Rodhe W (1949) Environmental requirements of fresh-water plankton algae. Symb Bot Ups X:149

Roelofs TD, Oglesby RT (1970) Ecological observations on the planktonic cyanophyte *Gloeotrichia echinulata*. Limnol Oceanogr 15:224–229

Rosenberg G, Paerl HW (1981) Nitrogen fixation by blue-green algae associated with the siphonous green seaweed *Codium decorticum*. Mar Biol 61:151–158

Rother JA, Aziz A, Hye Karim N, Whitton BA (1988) Ecology of deep-water rice-fields in Bangladesh 4. Nitrogen dixation by blue-green algal communities. Hydrobiologia 169:43–56

Rott E (1991) Oncoids from the summer-warm River Alz (Bavaria) – mophology and dominant cyanophytes. Algol Stud 64:469–482

Ruangsomboon S, Chidthaisong A, Bunnag B, Inthorn D, Harvey NW (2006) Lead (Pb^{2+}) removal from wastewater by the cyanobacterium *Calothrix marchica*. Kasetsart J (Nat Sci) 40:784–794

Ruiz-Ramírez R, Espinosa-Chávez F, Martínez-Jerónimo F (2005) Growth and reproduction of *Pomacea patula catemacensis* Baker, 1922 (Gastropoda: Ampullariidae) when fed *Calothrix* sp (Cyanobacteria). J World Aquacult Soc 36:87–95

Sabater S (1989) Encrusting algal communities in a Mediterranean river basin. Arch Hydrobiol 114:555–573

Sabater S, Guasch H, Romani A, Muñoz I (2000) Stromatolitic communities in Mediterranean streams: adaptations to a changing environment. Biodivers Conserv 9:379–392

Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol Biol Evol 19:101–109

Schäfer A, Stapf KRG (1978) Permian Saar-Nahe basin and recent Lake Constance (Germany): two environments of lacustrine algal carbonates. Spec Publ Int Assoc Sediment 2:83–107

Schlegel I, Doan NT, de Chazal N, Smith GD (1998) Antibiotic sensitivity of cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. J Appl Phycol 10:471–479

Schneider SC, Lindstrøm E-A (2011) The periphyton index of trophic status PIT: a new eutrophication metric based on non-diatomaceous benthic algae in Nordic rivers. Hydrobiologia 685:143–156

Schwenender S (1894) Zur Wachstumgeschichte der Rivularien. Sber Preuss Akad Wissensch, Berlin, pp 951–961

Shalini IM, Dhar DW, Gupta RK (2008) Phylogenetic analysis of cyanobacterial strains of genus-*Calothrix* by single and multiplex randomly amplified polymorphic DNA-PCR. World J Microbiol Biotechnol 24:927–935

Shehata FHA, Whitton BA (1981) Field and laboratory studies on blue-green algae from aquatic sites wutg high zinc levels. Verh Int Verein Limnol 21:1466–1471

Sigler WV, Bachofen R, Zeyer J (2003) Molecular characterization of endolithic cyanobacteria inhabiting exposed dolomite in central Switzerland. Environ Microbiol 5:618–627

Sihvonen LM, Lyra C, Fewer DP, Rajaniemi-Wacklin P, Lehtimäki JM, Wahlsten M, Sivonen K (2007) Strains of the cyanobacterial genera *Calothrix* and *Rivularia* isolated from the Baltic Sea display cryptic diversity and are distantly related to *Gloeotrichia* and *Tolypothrix*. FEMS Microbiol Ecol 61:74–84

Sinclair C, Whitton BA (1977a) Influence of nutrient deficiency on hair formation in the Rivulariaceae. Br Phycol J 12:297–313

Sinclair C, Whitton BA (1977b) Influence of nitrogen source on morphology of Rivulariaceae (Cyanophyta). J Phycol 13:335–340

Singh RN, Tiwari DN (1970) Frequent heterocyst germination in the blue-green alga *Gloeotrichia ghosei* Dingh. J Phycol 6:172–176

Skácelová O (2006) *Dichothrix ledereri* sp. nova, a new cyanobacterium from old coal/mining deposits, and occurrence of the genus *Dichothrix* (Cyanobacteria, Nostocales) in the Czech Republic. Algol Stud 121:1–21

Skulberg OM (2000) Microalgae as a source of bioactive molecules – experience from cyanophyte research. J Appl Phycol 12:341–348

Smith GD, Doan NT (1999) Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. J Appl Phycol 11:337–344

Smith RV, Peat A (1967) Comparative structure of the gas-vacuoles of blue-green algae. Arch Mikrobiol 57:111–122

Snoeijs P, Murasi LK (2004) Symbiosis between diatoms and cyanobacterial colonies. Vie Milieu 54:163–170

Spodniewska I (1971) The influence of experimental increase of biomass of the blue-green algae *Gloeotrichia echinulata* (Smith) Richter on phytoplankton production. Ekol Pol 19:475–483

Srivastava AK, Bhargava P, Kumar A, Rai LC, Neilan BA (2009) Molecular characterization and the effect of salinity on cyanobacterial diversity in the rice fields of Eastern Uttar Pradesh, India. Saline Syst 5/1/4:17. doi:10.1186/1746-1448-5-4

Stewart WDP (1967) Nitrogen turnover in fresh and marine habitats. II. Use of ^{15}N in measuring nitrogen fixation in the field. Ann Bot N.S. 31:385–407

Taton A, Grusibic S, Ertz D, Hodgson DA, Piccardi R, Biondi N, Tredici MR, Mainini M, Losi D, Marinelli F, Wilmotte A (2006) Polyphasic study of Antarctic cyanobacterial strains. J Phycol 42:1257–1270

Thajuddin N, Muralitharan G, Sundaramoorthy M, Ramamoorthy R, Ramachandran S, Akbarsha MA, Gunasekaran M (2010) Morphological and genetic diversity of symbiotic cyanobacteria from cycads. J Basic Microbiol 50:254–265

Theivendirarajah K, Jeyaseelan K (1977) The ingestion of blue-green algae by mosquito larvae. Ceylon J Sci Biol Sci 12:156

Thomazeau S, Houdan-Fourmont A, Couté A, Duval C, Couloux A, Rousseau F, Bernard C (2010) The contribution of sub-Saharan African strains to the phylogeny of cyanobacteria: focusing on the Nostocaceae (Nostocales, Cyanobacteria). J Phycol 46:564–579

Trautman DA, Borowitzka MA (1999) Distribution of the epiphytic organisms on *Posidonia australis* and *P. sinuosa,* two seagrasses with differing leaf morphology. Mar Ecol Prog Ser 179:215–229

Turner BL, Baxter R, Ellwood NTW, Whitton BA (2003a) Seasonal phosphatase activities of mosses from Upper Teesdale, northern England. J Bryol 25:189–200

Turner BL, Baxter R, Whitton BA (2003b) Nitrogen and phosphorus in soil solutions and drainage streams in Upper Teesdale, northern England: implications of organic compounds for biological nutrient limitation. Sci Total Environ 314–316C:153–170

Tymowski RG, Duthie HC (2000) Life strategy and phosphorus relations of the cyanobacterium *Gloeotrichia echinulata* in an oligotrophic Precambrian Shield lake. Arch Hydrobiol 148:321–332

Uku J, Björk M, Bergman B, Diez B (2007) Characterization and comparison of prokaryotic epiphytes associated with three East African seagrasses. J Phycol 43:768–779

Vaidya HM (1989) Comparative studies on *Calothrix* isolates from Nepalese rice-fields. M.Sc. thesis, University of Durham, Durham

Van Geel B, de Lange L, Wiegers J (1984) Reconstruction and interpretation of the local vegetational succession of a Lateglacial deposit from Usselo (The Netherlands), based on the analysis of micro- and macrofossils. Acta Bot Neerl 33(4):535–546

Van Wagoner RM, Drummond AK, Jeffrey LC, Wright JLC (2007) Biogenetic diversity of cyanobacterial metabolites. Adv Appl Microbiol 61:89–217

Vuorio K, Meili M, Sarvala J (2009) Natural isotopic composition of carbon($\delta^{13}C$) correlates with colony size in the planktonic cyanobacterium *Gloeotrichia echinulata*. Limnol Oceanogr 54(3):925–929

Webber EE (1967) Bluegreen algae from a Massachusetts salt marsh. Bull Torrey Bot Club 94:99–106

Weckesser J, Hofmann K, Jürgens EJ, Whitton BA, Raffelberger B (1988) Isolation and chemical analysis of the sheaths of the filamentous cyanobacteria *Calothrix parietina* and *C. scopulorum*. J Gen Microbiol 134:629–634

West W (1912) Fresh-water algae. In: Clare Island survey. Proc R Irish Acad XXXI(Pt 16):1–62

Whitton BA (1987) The biology of Rivulariaceae. In: Fay P, Van Baalen C (eds) The Cyanobacteria – a comprehensive review. Elsevier, Amsterdam, pp 513–534, 543 pp

Whitton BA (1988) Hairs in eukaryotic algae. In: Round FE (ed) Algae and the aquatic environment contributions in honour of J.W.G.Lund. Biopress, Bristol, pp 446–460, 460 pp

Whitton BA (1989) Genus I. *Calothrix* Agardh 1824. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3, 1st edn. Williams & Wilkins, Baltimore, pp 1791–1794

Whitton BA (2007) The blue-green algae of Clare Island, Co. Mayo, Ireland. In: New Survey of Clare Island, vol 6: the freshwater and terrestrial algae. Royal Irish Academy, Dublin, pp +141–178, 254 pp

Whitton BA (2008) Cyanobacterial diversity in relation to the environment. In: Evangelista V, Barsanti L, Frassanito AM, Gualtieri P, Passarelli V (eds) Algal toxins, nature, occurrence, effect and detection. Springer, Dordrecht, pp 17–35, 399 pp

Whitton BA (2009) Phosphorus status and the naming of freshwater algae. Algas 41:20–22

Whitton BA (2011) Phylum cyanobacteria (Cyanophyta). In: John DM, Whiton BA, Brook AJ (eds) The freshwater algal flora of the British Isles, 2nd edn. Cambridge University Press, Cambridge, pp 31–158, 878 pp

Whitton BA, Neal C (2010) Organic phosphate in UK rivers and its relevance to algal and bryophyte surveys. Int J Limnol 47:1–8

Whitton BA, Potts M (eds) (2000) The ecology of cyanobacteria. Their diversity in time and space. Springer, Dordrecht, 669 pp

Whitton BA, Gale NL, Wixson BG (1981) Chemistry and plant ecology of zinc-rich wastes dominated by blue-green algae. Hydrobiologia 83:331–341

Whitton BA, Grainger SLJ, Harris N (1986) Blue heterocysts in the Rivulariaceae. Br Phycol J 21(3):338–339

Whitton BA, Aziz A, Francis P, Rother JA, Simon JW, Tahmida ZN (1988a) Ecology of deepwater rice-fields in Bangladesh. 1. Physical and chemical environment. Hydrobiologia 169:3–22

Whitton BA, Aziz A, Rother JA (1988b) Ecology of deepwater rice-fields in Bangladesh. 3. Associated algae and macrophytes. Hydrobiologia 169:31–42

Whitton BA, Grainger SLJ, Hawley GRW, Simon JW (1991) Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb Ecol 21:85–98

Whitton BA, Yelloly JM, Christmas M, Hernández I (1998) Surface phosphatase activity of benthic algal communities in a stream with highly variable ambient phosphate concentrations. Verh Int Verein Limnol 26:967–972

Whitton BA, Al-Shehri AH, Ellwood NTW, Turner BL (2005) Ecological aspects of phosphatase activity in cyanobacteria, eukaryotic algae and bryophytes. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. Commonwealth Agricultural Bureau, Wallingford, pp 205–241, 399 pp

Wickstrom CE, Castenholz W (1985) Dynamics of cyanobacterial and ostracod interactions in an Oregon hot spring. Ecology 66:1024–1241

Wilkinson CR, Sammarco PW, Trott LA (1985) Seasonal and fish grazing effects on rates of nitrogen fixation on coral reefs (Great Barrier Reef, Australia). In: Proceedings of the 5th international coral reef congress, vol 4l, Tahiti, pp 61–65

Wilmotte A, Herdman M (2001) Phylogenetic relationships among the cyanobacteria based on 16S rRNA sequences. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 487–493, 721 pp

Wood P (1984) Structural and physiological studies on the effects of mineral deficiencies on the Rivulariaceae (Cyanobacteria), vols I and II. Ph D thesis (CNAA), Sunderland Polytechnic (now Sunderland University), UK

Wood P, Peat A, Whitton BA (1986) Influence of phosphorus status on fine structure of the cyanobacterium (blue-green alga) *Calothrix parietina*. Cytobios 47:89–99

Yelloly JM, Whitton BA (1996) Seasonal changes in ambient phosphate and phosphatase activities of the cyanobacterium *Rivularia atra* in intertidal pools at Tyne Sands, Scotland. Hydrobiologia 325:201–212

Zarmouh MM (2010) Antibacterial activity of *Rivularia* species and *Oscillatoria salina* collected from coastal region of Misurata, Libya. J Arab Soc Med Res 5(2):159–163

Zehnder A (1963) Kulturversuch mit *Gloeotrichia echinulata* (J. E. Smith) P. Richter. Schweiz Z Hydrol 25:65–83

Zhao-Liang X (1984) Investigation on the procaryotic microfossils from Gaoyuzhuang Formation, Jixian, North China. Acta Bot Sin 26(216–222):312–319

Cyanobacterial Symbioses

23

David G. Adams, Paula S. Duggan and Owen Jackson

Contents

D.G. Adams (✉) • P.S. Duggan • O. Jackson
Faculty of Biological Sciences, University of Leeds,
Leeds LS2 9JT, UK
e-mail: d.g.adams@leeds.ac.uk; owen.jackson@bis.gsi.gov.uk

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Summary

Cyanobacteria form symbiotic partnerships with a wide range of eukaryotic hosts including fungi, plants and animals such as sponges, ascidians and corals. They provide the host with fixed nitrogen and fixed carbon, and in return occupy relatively protected environments free from predation and environmental extremes. As well as being photoautotrophs, many cyanobacteria are capable of heterotrophy, enabling them to occupy symbiotic structures, such as the roots of plants, that receive little or no light and where photosynthetic hosts can supply them with fixed carbon. In all but a few cases cyanobacterial symbionts are capable of independent growth, but they frequently show significant morphological and physiological modifications when in symbiosis. Many cyanobacterial symbionts fix N_2 in specialised cells known as heterocysts and in many symbioses, notably those with plant hosts, the frequency of heterocysts is greatly elevated, as is the rate of N_2 fixation. A number of cyanobacterial symbioses are of major environmental significance as suppliers of fixed nitrogen to their surroundings. Some, such as the diatoms, can reach enormous populations in the oceans, whereas moss epiphytic associations are abundant in boreal forests, and cyanolichens are abundant in harsh environments where there are few other sources of fixed nitrogen.

23.1 Introduction

Cyanobacteria are found in symbiosis with a remarkable variety of hosts including plants, fungi, sponges and protists. In the majority of these symbioses the cyanobiont's contribution to the partnership is the products of N_2 fixation, enabling the hosts, such as plants and lichens, to occupy N limited environments. However, being photoautotrophs, cyanobacterial symbionts (cyanobionts) are also capable of supplying fixed carbon to non-photosynthetic hosts such as the fungi of lichens and *Geosiphon pyriformis*. Cyanobionts may also help to protect the host from excessive sunlight (e.g. sponges) or grazing (e.g. ascidians and isopod crustaceans). What the cyanobacteria gain is often less obvious, although protection from environmental extremes, predation and competition are all likely benefits. Photosynthetic hosts can also provide fixed carbon to the cyanobiont and this capacity for heterotrophy enables many cyanobionts to grow in host structures, such as the roots of cycads or the stem glands of *Gunnera*, that receive little or no light. All hosts are capable of independent growth if provided with the necessary nutrients, as are all cyanobionts, with the exception of those in *Azolla* and some diatoms. Within the wide variety of cyanobacterial symbioses there seems to be no correlation between the presumed evolutionary age of the symbiosis and the location of the cyanobiont (e.g. inter- or intra-cellular) within the host, its mode of transmission, or its importance to host well-being (Usher et al. 2007). In other words, the degree of integration between host and cyanobiont, and the mode of cyanobiont transmission are not good indicators of the evolutionary age of the symbiosis or its importance to the host.

A wide variety of cyanobacteria, both unicellular and filamentous, forms symbiotic associations. Perhaps the most common cyanobionts are from the genus *Nostoc* and possess two important characteristics – they fix N_2 in specialised cells known as heterocysts and they produced motile filaments known as hormogonia. The heterocyst provides the necessary microoxic environment for the functioning of the enzyme nitrogenase, which performs biological N_2 fixation and is highly sensitive to oxygen (Golden and Yoon 2003; Zhang et al. 2006; Flores and Herrero 2010). Unicellular or filamentous non-heterocystous N_2-fixing cyanobacteria have to employ alternative strategies to protect nitrogenase, such as the temporal separation of N_2 fixation and oxygenic photosynthesis. The hormogonium provides a motile phase in the otherwise sessile *Nostoc* life cycle and serves both as a means of dispersal and as the infective agent in many of the cyanobacterial symbioses with plants and fungi (Meeks et al. 2002; Meeks and Elhai 2002; Meeks 2009). Many plant hosts secrete chemical signals that both stimulate hormogonia production and serve as chemoattractants to guide the hormogonia to the symbiotic structures. Once infected, some, and perhaps all plant hosts secrete hormogonia-repressing factors to ensure that the cyanobiont returns to vegetative growth and produces heterocysts to fix N_2 for the host.

This chapter deals with the literature from 2000 onwards. For coverage of the earlier literature the reader is directed to Adams (2000) and the many reviews and chapters listed here. Earlier research on cyanobacterial symbioses was largely concerned with the more experimentally amenable of the plant associations such as those with *Gunnera*, *Azolla* and bryophytes such as hornworts and liverworts. More recently there has been increasing interest in associations such as sponges and mosses.

23.2 The Symbioses and Their Environmental Impact

23.2.1 Plants

Cyanobacterial-plant symbioses are ancient associations believed to have evolved around 500 million years ago (Raven 2002a, b; Bergman et al. 2008a, b), a hypothesis supported by the discovery of fossil evidence of cyanobacteria

inside 400 million year old land plants (Taylor and Krings 2005; Krings et al. 2009); these cyanobacteria were non-heterocystous and probably more closely resembled Oscillatoriales rather than the Nostocales that typically enter into existing plant associations. Warm and moist environments, supporting close association of plants and cyanobacteria, probably stimulated the evolution of cyanobacterial symbioses (Usher et al. 2007). These conditions are thought to have favoured enhanced plant growth, thereby increasing the demand for N. Additionally, for hormogonia (the infective agents in most cyanobacteria-plant symbioses; Sect. 23.4.1.2), to remain motile they require the presence of some moisture, such as a thin film of water (Usher et al. 2007). Although in most cases the cyanobiont is still capable of growth away from the host, an exception is the water fern *Azolla* in which the adaptations of the cyanobiont are more extreme and it is no longer capable of independent growth, indicating that some biological feature critical for the free-living state has been lost during the millions of years of co-evolution with its host. The cyanobiont might even be evolving towards being a N_2 fixing organelle in a manner akin to that believed to have given rise to the chloroplast (Ekman et al. 2008; Ran et al. 2010).

23.2.1.1 Loose Associations

Although most of the symbioses described in this chapter involve cyanobionts living within the tissues or the cells of the host, there are many looser associations in which cyanobacteria grow as epiphytes on the surface of plants. With the exception of the cyanobacteria-moss epiphytic associations, which are dealt with later, these associations are mostly poorly studied. They are probably common, although the degree of benefit obtained by the epiphytic cyanobacterium and the plant is often unclear.

Epiphytic growth of N_2 fixing *Nostoc*, *Gloeotrichia*, *Anabaena*, *Calothrix* and *Cylindrospermum* has been reported for rice plants and duckweed (see Adams 2000) and the unicellular *Chamaesiphon* spp. and *Xenococcus kerneri*, which have not yet been checked for possible N_2 fixation, are common on epilithic algae and submerged mosses (Lindstrøm et al. 2004; Kučera et al. 2005). Cyanobacteria are also common epiphytes on the pneumatophores of mangroves (Steinke et al. 2003). In rice fields in Spain the macroalga *Chara vulgaris* harbours N_2 fixing epiphytic cyanobacteria belonging to the heterocystous genera *Calothrix*, *Nostoc* and *Anabaena* (Ariosa et al. 2004). *Chara* is found in rice fields world-wide and is generally thought to be a weed, but its N_2 fixing epiphytic cyanobacteria may contribute to soil fertility (Ariosa et al. 2004). Various cyanobacteria, including *Nostoc*, *Scytonema* and *Calothrix*, have also been found on the aerial roots of the epiphytic orchids *Acampe papillosa*, *Phalaenopsis amabilis* and *Dendrobium moschatum* and the substrate roots of *A. papillosa* and *D. moschatum* although it is unclear if the orchids benefit from fixed N produced by the cyanobacteria (Tsavkelova et al. 2001, 2003a, b). A potentially endophytic cyanobiont, resembling the unicellular *Dactylococcopsis acicularis*, has been reported in the roots of the orchid *Spathoglotis plicata* (Untari et al. 2009).

Epiphytic growth of cyanobacteria is also common in the marine environment. For example, the chlorophyll *d*-containing cyanobacterium *Acaryochloris marina* is found as an epiphyte on the marine red macroalga *Ahnfeltiopsis flabelliformis* (Murakami et al. 2004) and on green and brown marine macroalgae (Ohkubo et al. 2006). Another marine red macroalga, *Acanthophora spicifera*, can become covered by epiphytic *Lyngbya* (Fong et al. 2006). *A. spicifera* formed blooms on some Eastern Pacific reefs following coral mortality resulting from the 1997–1998 El Nino Southern Oscillation. The alga lacking the cyanobacterial epiphyte is highly palatable to herbivores, but the cyanobacterium greatly reduces herbivory, presumably by the production of chemical defences, and this increases the ability of the alga to become dominant (Fong et al. 2006).

Lyngbya spp. are also found as epiphytes on seagrasses in Florida Bay where addition of P stimulates their growth and that of co-occurring red algal epiphytes (Armitage et al. 2006; Frankovich et al. 2009). Bacterial epiphytes on seagrasses such as *Thalassia testudinum* may obtain organic carbon from cyanobacterial and algal epiphytes rather than from the seagrass itself (Williams et al. 2009). Cyanobacteria are common epiphytes on seagrasses, and in highly oligotrophic seas such as the Gulf of Elat they can make significant contributions to the N required for primary productivity in the seagrass beds (Pereg-Gerk et al. 2002). Cyanobacterial epiphytes on the leaves of three seagrasses *Thalassodendron ciliatum*, *Thalassia hemprichii* and *Cymodocea rotundata* from two Kenyan coastal sites show enough distinct differences between the seagrass species to suggest that there may be some host specificity, particularly in *C. rotunda* (Uku et al. 2007). On the same seagrasses low nutrient levels favour the growth of cyanobacterial over algal epiphytes (Uku and Bjork 2001). Heterocystous cyanobacteria such as *Calothrix* and *Anabaena* and other potential N_2 fixers may enable *C. rotunda* to maintain a rapid growth rate at a low-nutrient, N-limited site, where seagrasses lacking these cyanobacteria are disadvantaged. In this way N_2 fixation by epiphytic cyanobacteria may contribute to the productivity of seagrass beds (Hamisi et al. 2009).

Compared with the leaves of aquatic plants, the leaf surface (phyllosphere) of land plants is a much harsher environment for cyanobacteria, but they can be found in the phyllosphere in tropical rainforests where the humidity is high. For example, in a Costa Rican lowland rainforest heterocystous *Nostoc*, *Fischerella* and *Tolypothrix* are found on leaf surfaces, often in association with epiphytic bryophytes

(Fürnkranz et al. 2008). Although found at comparatively low abundance, these cyanobacteria are the major component of the N_2 fixing bacterial community and may provide significant N input into this rainforest ecosystem. However, not all epiphytic cyanobacteria are beneficial to the "host". For example, *Brasilonema octagenarum* strain UFV-E1 (Scytonemataceae) forms a dense mat on the surface of the leaves of *Eucalyptus grandis* (Aguiar et al. 2008). The cyanobacterial mat blocks light, causing a reduction in photosynthesis in the leaves, and interfering with stomatal gas exchange, so decreasing CO_2 assimilation.

23.2.1.2 Bryophytes (Mosses, Hornworts and Liverworts)

The bryophytes, encompassing the liverworts (Hepaticae), the hornworts (Anthocerotae) and the mosses (Musci), are small, non-vascular land plants, some of which form epiphytic or endophytic (Figs. 23.1 and 23.2) associations with cyanobacteria (Adams 2002a, b; Meeks 2003; Solheim et al. 2004; Adams et al. 2006; Adams and Duggan 2008; Bergman et al. 2007a, 2008a), primarily of heterocyst-forming genera *Nostoc*, *Stigonema* and *Calothrix*. In the mosses the cyanobacteria are mostly epiphytic, often being found between the stem and the leaf (Solheim and Zielke 2002; Solheim et al. 2004; Gentili et al. 2005), with the exception of two *Sphagnum* species in which they occupy water-filled, dead (hyaline) cells, where they are thought to be protected from the acidic bog environment (Solheim and Zielke 2002). Cyanobacteria growing on the moss leaf surface are thought to be protected by alkaline substances secreted by the leaf (Belnap 2001). In *Sphagnum*, the acidity of the bog environment may be the factor that determines whether cyanobacteria grow epiphytically (higher pH) or intracellularly within hyaline cells (lower pH; Solheim and Zielke 2002). These moss associations with N_2 fixing cyanobacteria can supply most of the combined nitrogen in local ecosystems in the Arctic, the Antarctic and boreal forest regions (Zielke et al. 2002, 2005; Solheim and Zielke 2002; Nilsson and Wardle 2005; DeLuca et al. 2008; Stewart et al. 2011). Examples of other forest systems where they may be a major source of biologically-derived nitrogen include ones from tropical forests (Cusack et al. 2009) and New Zealand (Menge and Hedin 2009). This topic is discussed further in Chap. 10.

Fig. 23.1 The liverwort *Blasia pusilla* collected from the wild, showing the dark *Nostoc* colonies (~0.5–1.0 mm in diameter) bordering the thallus midrib. (Reproduced with permission from Adams 2000)

Mosses

Cyanobacteria-moss associations may be especially important in boreal forests where up to 80% of the ground cover can consist of the feather moss *Pleurozium schreberi* with its epiphytic cyanobacteria (Zackrisson et al. 2004; Gentili et al. 2005; Nilsson and Wardle 2005; Lagerström et al. 2007; DeLuca et al. 2007, 2008). Indeed, *P. schreberi* is one of the most common mosses on earth (DeLuca et al. 2002) and in boreal forests feather moss growth can exceed that of trees (Bond-Lamberty and Gower 2007). The mosses *Hylocomium splendens* and *Ptillium crista-castrensis* also associate with N_2-fixing cyanobacteria (Solheim et al. 2004; Houle et al. 2006; Zackrisson et al. 2009) and the moss *Sphagnum capillifolium* has even higher rates of N_2 fixation than *Pleurozium schreberi*, even when the two occur at the same site (Markham 2009). Recent work in old growth forests in British Columbia has shown that epiphytic moss-cyanobacteria associations may show nitrogen fixation rates even greater than those of moss carpets on the forest floor (Lindo and Whiteley 2011).

In alpine and arctic heath tundra in Sweden *Pleurozium schreberi* and *Hlyocomium splendens* are usually responsible for less than 5% of the ground cover, yet under patches of the common juniper this can be as high as 60–80%. These moss carpets have N_2 fixation rates of 150 µmol acetylene reduced m^{-2} day^{-1}, 10–15 times higher than in the open heath (DeLuca and Zackrisson 2007). This elevated N_2 fixation rate may result from the ability of the junipers to use their extensive root systems to scavenge P, resulting in raised P levels beneath the shrubs as a result of litter deposition. The presence of these moss "islands" in this alpine tundra can result in levels of N_2 fixation as high as 1.4 kg N ha^{-1} $year^{-1}$ (DeLuca and Zackrisson 2007). However, the N fixed by cyanobacteria epiphytic on feather moss may have a relatively low availability to the local ecosystem because the mosses are highly efficient

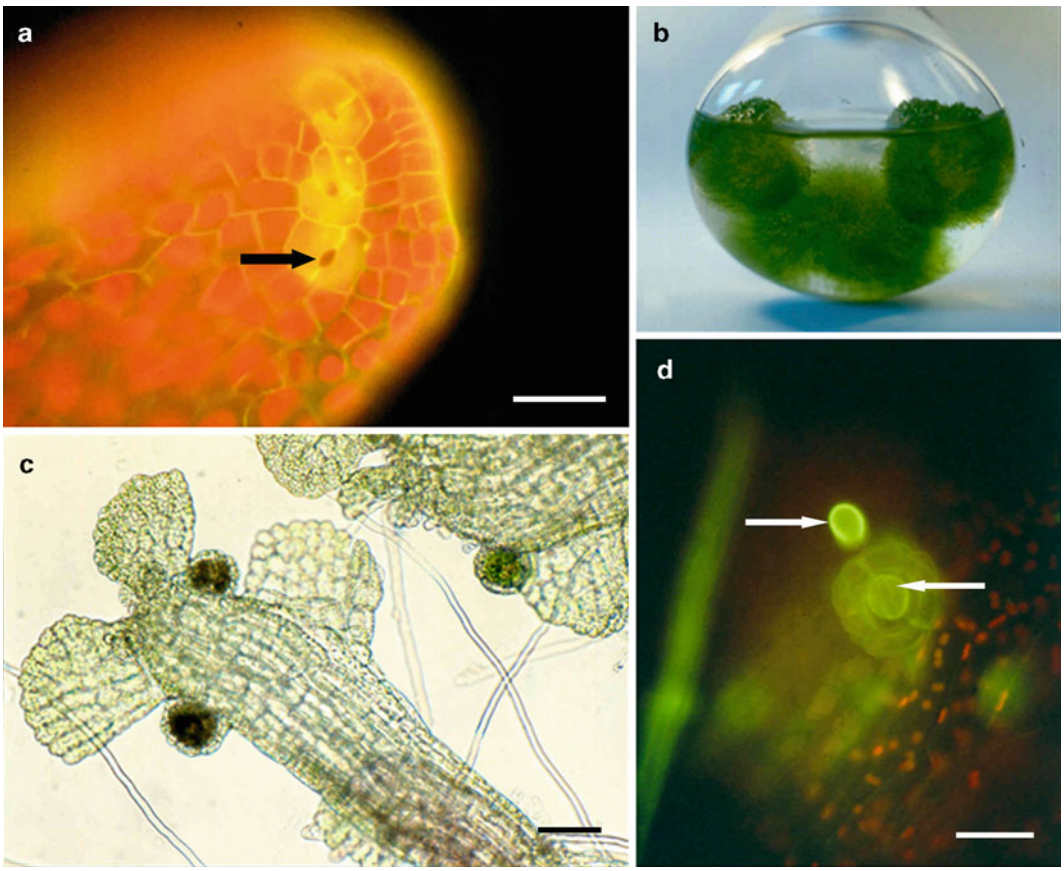

Fig. 23.2 The liverwort and hornwort symbioses. (**a**) Fluorescence micrograph of the hornwort *Phaeoceros* stained with calcofluor, showing the slit-like entrances (one of which is arrowed) through which hormogonia gain entry to the slime cavities beneath. (**b**) View of the underside of an Erlenmeyer flask containing the liverwort *Blasia pusilla* grown free of cyanobacteria in shaken liquid medium. (**c**) *Blasia pusilla* growing in liquid culture showing three auricles infected in the laboratory with two different *Nostoc* strains, one brown pigmented (the two auricles to the *left*) and the other blue-green. (**d**) Fluorescence micrograph of uninfected *Blasia* stained with calcofluor. A single auricle can be seen with one inner (lower arrow) and one outer (upper arrow) slime papilla. Bars 50 μm (Photographs (**a**) and (**d**) courtesy of S. Babic. (**a**) and (**d**) reproduced with permission from Adams 2000; (**b**) reproduced with permission from Adams 2002a; (**d**) reproduced with permission from Adams and Duggan 1999)

at retaining this N and the decomposition rate of dead feather moss tissue is very low (Lagerström et al. 2007).

N_2 fixation by cyanobacteria-moss associations is greatly influenced by existing environmental factors, such as water availability, and may be adversely affected by future changes such as ozone depletion and the resulting increases in UV-B radiation. For example, N_2 fixation rates in cyanobacteria-moss associations in the arctic are greatly reduced by 3–6 years exposure to artificially enhanced UV-B radiation, equivalent to a 15% depletion of the ozone layer (Solheim et al. 2002, 2006). Prolonged drought can also result in a decline in the N_2 fixation capacity of cyanobacteria-moss associations, whereas persistent moisture results in an increase (Gundale et al. 2009). This probably explains the frequent reports of decreased N_2 fixation rates in mid-summer when conditions are at their driest (DeLuca et al. 2002; Zackrisson et al. 2004).

N_2 fixation in cyanobacteria-moss associations is very sensitive to external combined N. For example, fertilization with ammonium nitrate can reduce or eliminate N_2 fixation (Zackrisson et al. 2004; DeLuca et al. 2007; Gundale et al. 2011) and reduce colonization of moss shoots by cyanobacteria (DeLuca et al. 2007). However, sensitivity to external N input varies between mosses. For example, the cyanobacteria-*P. schreberi* association seems to be more sensitive to external N input than the *H. splendens* association (Zackrisson et al. 2009). Deposition of canopy throughfall N onto moss carpets can also inhibit cyanobacterial N_2 fixation (DeLuca et al. 2008). This inhibition by available nitrogen may explain the gradual increase in N_2 fixation rates following recovery from fire, a process which may take hundreds of years (Zackrisson et al. 2004; DeLuca et al. 2008). Support for this comes from transplantation of moss carpets from early secondary successional boreal forest sites (up to 101 years since

the last fire and with high levels of available N) to late successional sites (241–356 years since the last fire and with low levels of N). The low N, late successional sites had high rates of N_2 fixation and high levels of cyanobacterial colonization of moss shoots, but moss carpets transplanted from these sites to early successional sites, with high levels of available N, showed a decline in N_2 fixation rates and cyanobacterial colonization after 12 months (DeLuca et al. 2007). Conversely, transfer of late successional moss carpets to early successional sites resulted in decreased N_2 fixation rates and a decrease in cyanobacterial colonization. Other aspects of the interactions between cyanobacteria and these feather mosses are discussed in Sect. 10.4.6.

Hornworts and Liverworts

In the hornworts, of which 13 genera have been described (Duff et al. 2007), endophytic cyanobacterial associations are ubiquitous (Renzaglia et al. 2007) and new ones, such as *Nothoceros superbus*, are still being found (Villarreal et al. 2007). By contrast, of more than 340 liverwort genera only four form cyanobacterial associations, two of which (*Blasia* and *Cavicularia*) are endophytic and two (*Marchantia* and *Porella*) epiphytic (Adams et al. 2006; Adams and Duggan 2008). The flattened gametophyte thallus of liverworts and hornworts is a few centimetres in length and symbiotic colonies can be seen as dark spots up to 0.5 mm in diameter (Fig. 23.1). The thallus is attached to the substrate by root-like rhizoids. Liverworts such as *Blasia* and hornworts such as *Anthoceros* and *Phaeoceros*, make excellent laboratory models for cyanobacteria-plant symbiosis because of the ease with which the host plant can be grown, free of its symbionts, in shaken liquid culture, and the symbiosis re-formed with the original or with novel cyanobacteria (Fig. 23.2b; Meeks 2003; Duckett et al. 2004; Adams and Duggan 2008).

23.2.1.3 Gymnosperms (Cycads)

Between 250 and 65 million years ago the cycads dominated the Earth's forests, but today their distribution is limited to subtropical and tropical regions of mostly the southern hemisphere, including Australia and South Africa (Brenner et al. 2003; Vessey et al. 2005). They are the most primitive of today's seed plants (gymnosperms), consisting of approximately 250 species within the order Cycadales (Vessey et al. 2005; Bergman et al. 2007a). These evergreen, palm-like plants vary in height from a few tens of centimetres to 20 m, with a trunk and a large tap root from which may develop two additional root types: lateral and coralloid. Coralloid roots (so-called because of their coral-like appearance; Fig. 23.3a) are produced by all cycad species and show negative geotropism, growing sideways and upwards towards the soil surface; they become infected with N_2 fixing cyanobacteria, primarily of the genus *Nostoc* (Costa and Lindblad 2002; Lindblad and Costa 2002; Vessey et al. 2005; Bergman et al. 2007a), which are visible as a dark blue-green band between the inner and outer cortex (Fig. 23.3b). Nitrogen fixation in cycads can contribute up to 18.8 kg N ha^{-1} $year^{-1}$ to the local N economy (see: Rai et al. 2000; Vessey et al. 2005).

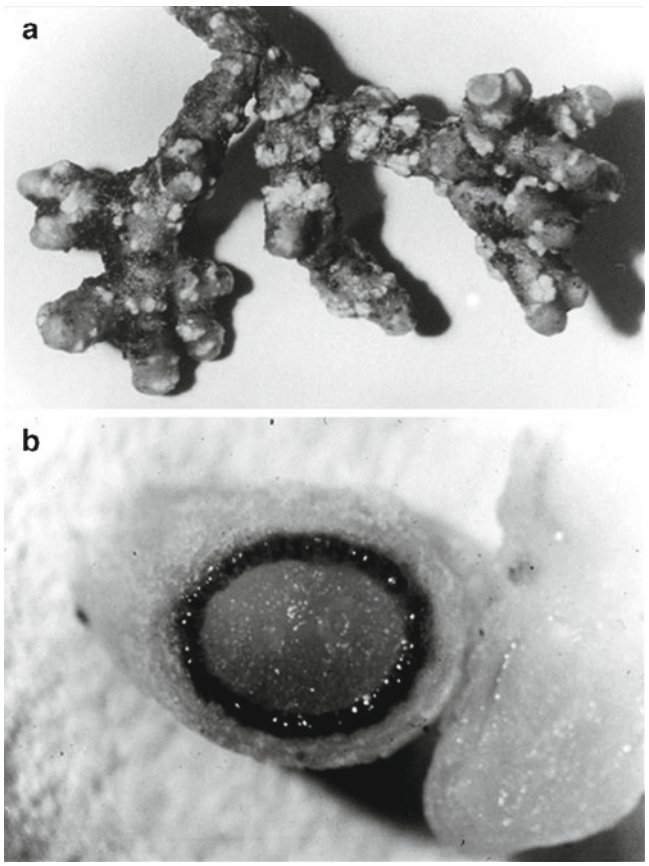

Fig. 23.3 The cycad-*Nostoc* symbiosis. (**a**) Cycad coralloid root, which is the site of cyanobacterial infection. (**b**) Transverse section of the root showing the dark cyanobacterial band between the inner and outer cortical layers of the root ((**a**) Reproduced with permission from Lindlbad et al. 1985; (**b**) reproduced with permission from Rai et al. 2000)

The cyanobionts of some cycad coralloid roots have been shown to produce the neurotoxic non-protein amino acid β-methylamino-L-alanine (BMAA; Cox et al. 2003) which may act as a deterrent to herbivory in cycads. BMAA was later shown to be produced by all known groups of free-living cyanobacteria (Cox et al. 2005; Banack et al. 2007). This neurotoxin was thought to be responsible for the high incidence of the progressive neurodegenerative disease amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS-PDC) in the Chamorro people on the island of Guam, who ingested the toxin through eating flying foxes (fruit bats) which had themselves eaten cycad seeds containing BMAA (Cox et al. 2003; Banack et al. 2006). However, this theory has remained controversial, not least because of difficulties in reliable separation and detection of BMAA. Some groups have confirmed the presence of BMAA

Fig. 23.4 *Gunnera manicata*. (**a**) Young plant with two large flower spikes. Inset: Red pigmented fronds cover the crown of the plant (hidden in the large image), where new leaves and new stem glands develop. (**b**) Vertical cross-section of a rhizome. Cyanobacterial colonies (0.5–2 cm in diameter) can be seen as green patches around the periphery of the rhizome. New leaves will develop in the region between the two leaf petioles at the top of the image, which is an area covered by red fronds (see inset in **a**). New stem glands form close to the base of each newly-developing leaf petiole and subsequently become infected by *Nostoc*. (Photos: Owen Jackson)

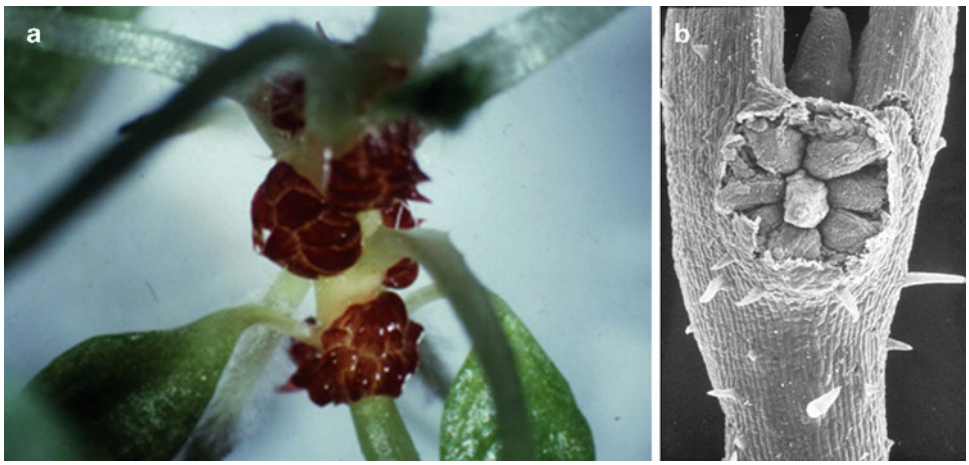

Fig. 23.5 *Gunnera* stem glands. (**a**) *Gunnera* seedling showing the red stem glands at the base of the leaf petioles. The glands are the entry point for cyanobacteria. (**b**) Scanning electron micrograph of a *Gunnera chilensis* gland showing the arrangement of papillae. Hormogonia gain entry into the internal stem gland tissue by migrating down the channels between the papillae. ((**a**) Reproduced with permission from Adams et al. 2006; (**b**) reproduced with permission from Bergman et al. 1992)

in cyanobacteria and cycad seeds (Esterhuizen and Downing 2008; Spáčil et al. 2010). However, contradictory results have been obtained when samples have been analysed by LC-MS/MS without prior derivatisation of samples (Rosén and Hellenäs 2008; Li et al. 2010; Krüger et al. 2010).

23.2.1.4 Angiosperms (*Gunnera*)

The symbiosis between *Nostoc* and *Gunnera* is unique for two reasons – it is the only symbiosis between an angiosperm (flowering plant) and a cyanobacterium, and it is the only one in which the cyanobiont is intracellular (Bergman 2002; Bergman and Osborne 2002; Bergman et al. 2007a). The cyanobiont is found inside mucus-secreting glands on the plant stem at the base of each leaf petiole (Figs. 23.4 and 23.5). The *Gunnera* genus consists of around 50 species that vary in size from creeping forms with leaves 1–10 cm across, to rhizomatous plants with leaves several metres across (such as *G. manicata*; Fig. 23.4a). The *Gunnera* cyanobiont may constitute as little as 1% of the plant mass yet can supply the entire N requirements of even

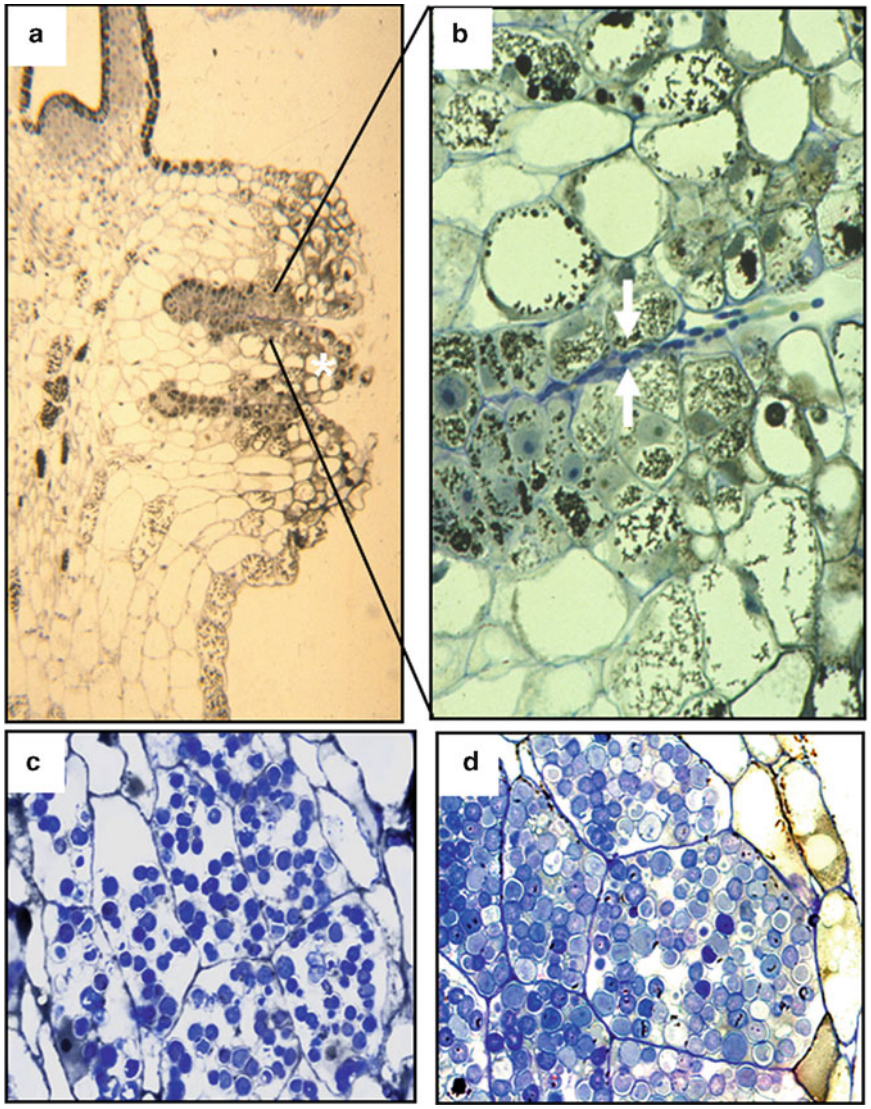

Fig. 23.6 *Gunnera* stem gland structure and infection. (**a**) Cross section of the stem gland showing three papillae separated by the channels that provide the route of infection into the gland tissues. (**b**) Close-up of one of the channels in (**a**) showing hormogonia (stained blue; arrows) migrating towards the inner parts of the gland. (**c**) *Gunnera* cells infected with cyanobacterial filaments (stained blue). At this early stage the filaments have a very low frequency of heterocysts, whereas at later stages (**d**) the heterocyst frequency increases greatly. (Reproduced with permission from Johansson and Bergman 1992)

the largest plants (Bergman et al. 2007a). The plants have a fossil record dating back 70–90 million years, making them the oldest of the angiosperms (Wikström et al. 2001; Raven 2002a). They were once only found in warm, wet equatorial regions such as South America, South East Africa, Madagascar and the Philippines, but now commonly appear in temperate climates such as Northern Europe, in suitably wet conditions (Osborne and Sprent 2002). The ecology, taxonomy and biogeography of the plant is reviewed elsewhere (Wanntorp and Wanntorp 2003; Fuller and Hickey 2005) (Fig. 23.6).

The *Nostoc-Gunnera* symbiosis appears to be mutually beneficial, in that the plant receives fixed nitrogen from the cyanobacterium (Uheda and Silvester 2001; Bergman and Osborne 2002; Bergman 2002), and while benefits to the cyanobacterium are less clear, it is likely that it is provided with an uncompetitive ecological niche, protection from predation and environmental extremes including desiccation (Badger et al. 2006), and also with fixed carbon from the plant (Black et al. 2002), possibly in the form of fructose (Parsons and Sunley 2001; Ekman et al. 2006). The relationship also appears to be facultative, in that both the plant and the cyanobiont can be cultured separately (Chiu et al. 2005). However, *Gunnera* only thrives when the cyanobiont is present; indeed all *Gunnera* plants in the wild contain *Nostoc* as an intracellular cyanobiont.

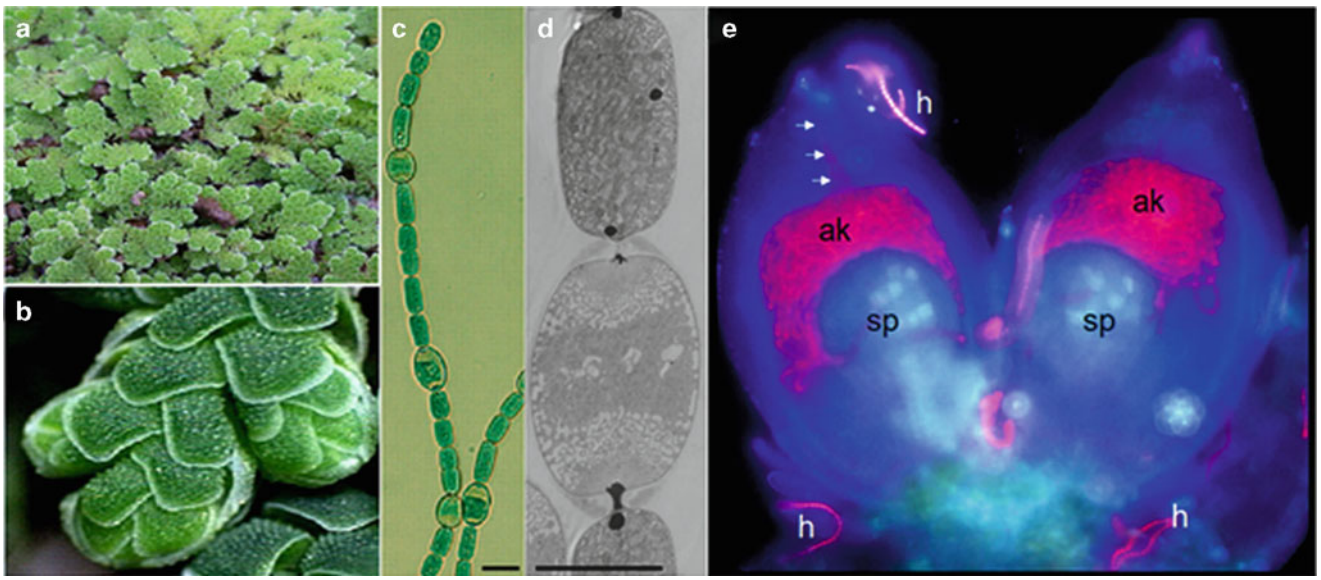

Fig. 23.7 The water fern *Azolla*. (**a**) View from above of *Azolla filliculoides* floating on the water surface. (**b**) View of an *Azolla* branch showing the overlapping dorsal lobes of the leaves which contain the cyanobionts. (**c**) Light micrograph of the *Azolla* cyanobiont with the large heterocysts clearly visible. (**d**) Transmission electron micrograph of a thin, longitudinal section of a cyanobiont filament showing a heterocyst (centre) with a vegetative cell on either side. (**e**) Fluorescence micrograph of a pair of megasporocarps (blue) which become infected with cyanobacteria when the motile hormogonia (*h*) on the surface, enter via channels (arrows). Once inside the megasporocarp the cells of the hormogonia convert into akinetes (ak) which can be seen as the intensely fluorescing area above the megaspores (sp). These akinetes provide the inoculum for the next generation of the fern, so maintaining the continuity of the symbiosis. Bars 5 μm (**c**), 5 μm (**d**). (Reproduced with permission from Ran et al. 2010)

23.2.1.5 Pteridophytes (*Azolla*)

Azolla consists of small (generally no greater than 3–4 cm) triangular or polygonal-shaped free-floating water ferns (Fig. 23.7a, b; Lechno-Yossef and Nierzwicki-Bauer 2002; van Hove and Lejeune 2002a, b; Bergman et al. 2007a, b). The genus *Azolla* comprises six or seven extant species traditionally grouped into two Sections, Azolla (synonymous with Euazolla) and Rhizosperma (Pabby et al. 2004a; Reid et al. 2006; Metzgar et al. 2007; Papaefthimiou et al. 2008b), primarily based on the characteristics of the reproductive structures. They are found worldwide from temperate to tropical climates on the surface of still or slow-moving bodies of freshwater such as ponds, paddy fields, ditches and marshes. *Azolla* coexists in mutual association with heterocyst-forming diazotrophic cyanobacteria and other eubacteria which are found in a cavity in the upper lobe of each leaf (Fig. 23.7). The *Azolla-Anabaena* symbiosis is the only example of a hereditary plant-cyanobacterial association in which the cyanobiont is transferred from one generation to the next. *Azolla* has wide agronomic and environmental applications including use as a green manure in rice cultivation (Vaishampayan et al. 2001; Bergman et al. 2007a, b), as a supplemental animal feed, in mosquito control, and in more recent years its potential for the removal of heavy metals from industrial effluent has been explored (van Hove and Lejeune 2002a, b; Choudhury and Kennedy 2004; Bennicelli et al. 2004; Tel-Or and Forni 2011). *Azolla*'s propensity for rapid growth also carries a disadvantage in some parts of the world where the plant is often regarded as a weed (Pabby et al. 2004b; Hashemloian and Azimi 2009).

Azolla has been used for centuries as a biological fertiliser in rice agriculture in China and other Far East countries and it has also been applied to enhance other crops, including bananas, wheat, tomato and taro (see: Vaishampayan et al. 2001; van Hove and Lejeune 2002a, b; Pabby et al. 2004a; Choudhury and Kennedy 2004; Franche et al. 2009). As well as nitrogen, *Azolla* also enriches soil fertility by supplying organic carbon, phosphorus and potassium (Pabby et al. 2004b). However, use of the crop has declined to less than 2% of the world's rice production, perhaps because its use is labour-intensive and the plant is susceptible to insect attack as well as being relatively sensitive to extremes of temperature and light intensity (Vaishampayan et al. 2001; Pabby et al. 2004b).

23.2.2 Fungi

23.2.2.1 Lichens

Lichens are stable symbiotic associations between a fungus (the mycobiont, which is usually an ascomycete) and a photosynthetic partner (the photobiont), which is a green alga or

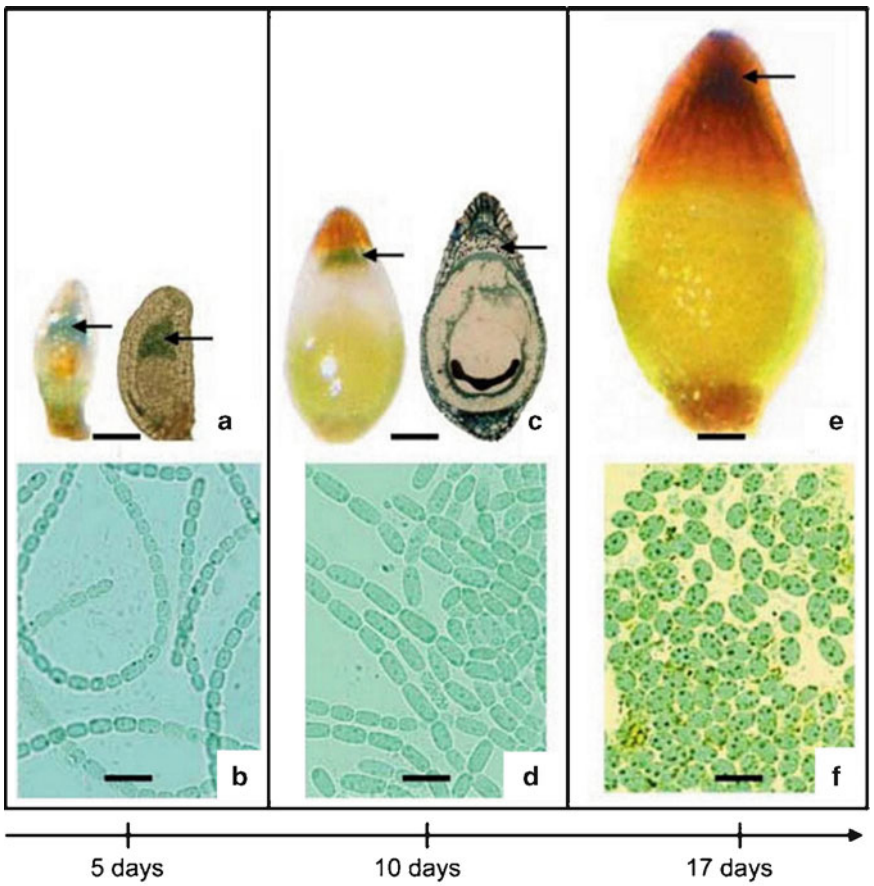

Fig. 23.8 Developmental sequence of the *Azolla microphylla* megasporocarp and its cyanobacterial colony. (**a**), (**c**) and (**e**) show a developmental sequence of the megasporocarp containing a cyanobacterial colony (arrow) in the indusium chamber above the megaspore. In (**a**) and (**c**) the intact megasporocarp is shown to the left and a semi-thin section to the right. (**b**), (**d**) and (**f**) show the morphology of the cyanobacteria in the megasporocarp represented by the developmental sequence shown in (**a**), (**c**) and (**e**) respectively. At 5 days (**b**) the cyanobacteria are mostly in the form of non-heterocystous hormogonial filaments. By 10 days (**d**) most cells have converted to large, elongated pro-akinetes. By 17 days (**f**) cells have developed into mature akinetes (a form of spore) containing numerous cyanophycin (nitrogen storage) granules. Bars 100 μm (**a**, **c**, **e**); 10 μm (**b**, **d**, **f**). (Reproduced with permission from Zheng et al. 2009b)

a cyanobacterium (Figs. 23.9 and 23.10; Sanders 2001, 2006; Rikkinen 2002; Rai and Bergman 2002; Oksanen 2006; Bergman et al. 2007a; Lücking 2008). Although not normally considered part of the symbiosis, bacterial communities growing as biofilms on the fungal surface may also be of importance (Grube et al. 2009), as may bacterial communities found within lichens (Bates et al. 2011). Of the 15,000–20,000 species of lichen, approximately 10% contain a cyanobacterium as the sole photobiont, and about 3% are so-called tripartite lichens which contain both a cyanobacterium (as the minor photobiont) and a green alga as the major photobiont (Rikkinen 2002; DePriest 2004; Adams et al. 2006; Bergman et al. 2007a). Of the nearly 20% of all fungi that form lichens, the vast majority are ascomycetes. Most cyanobionts are from the genus *Nostoc*, although members of other heterocystous and even unicellular genera are also involved (Rikkinen 2002). New cyanolichens and new cyanobionts are still being identified (Schultz et al 2000; Bjerke et al. 2003a; Grube 2005; Casamatta et al. 2006; Lücking et al. 2009) and this will no doubt continue. Some so-called cyanotrophic green algal lichens form either facultative or obligate associations with free-living N_2 fixing cyanobacteria, usually *Stigonema* or *Gloeocapsa*, presumably to access some of the N_2 they fix (Rikkinen 2002). Ascomycetes that obtain nutrients from cyanobacteria, often living within cyanobacterial colonies without forming a well-defined thallus, are also common although poorly understood (Rikkinen 2002).

Although the fossil record for lichens is poor (Rikkinen 2002), there is some evidence that lichen-like interactions between fungi and cyanobacteria or algae may have occurred over 600 million years ago, possibly in a shallow marine environment, long before vascular plants began to colonise the land (Yuan et al. 2005). Fossils identified as lichens have

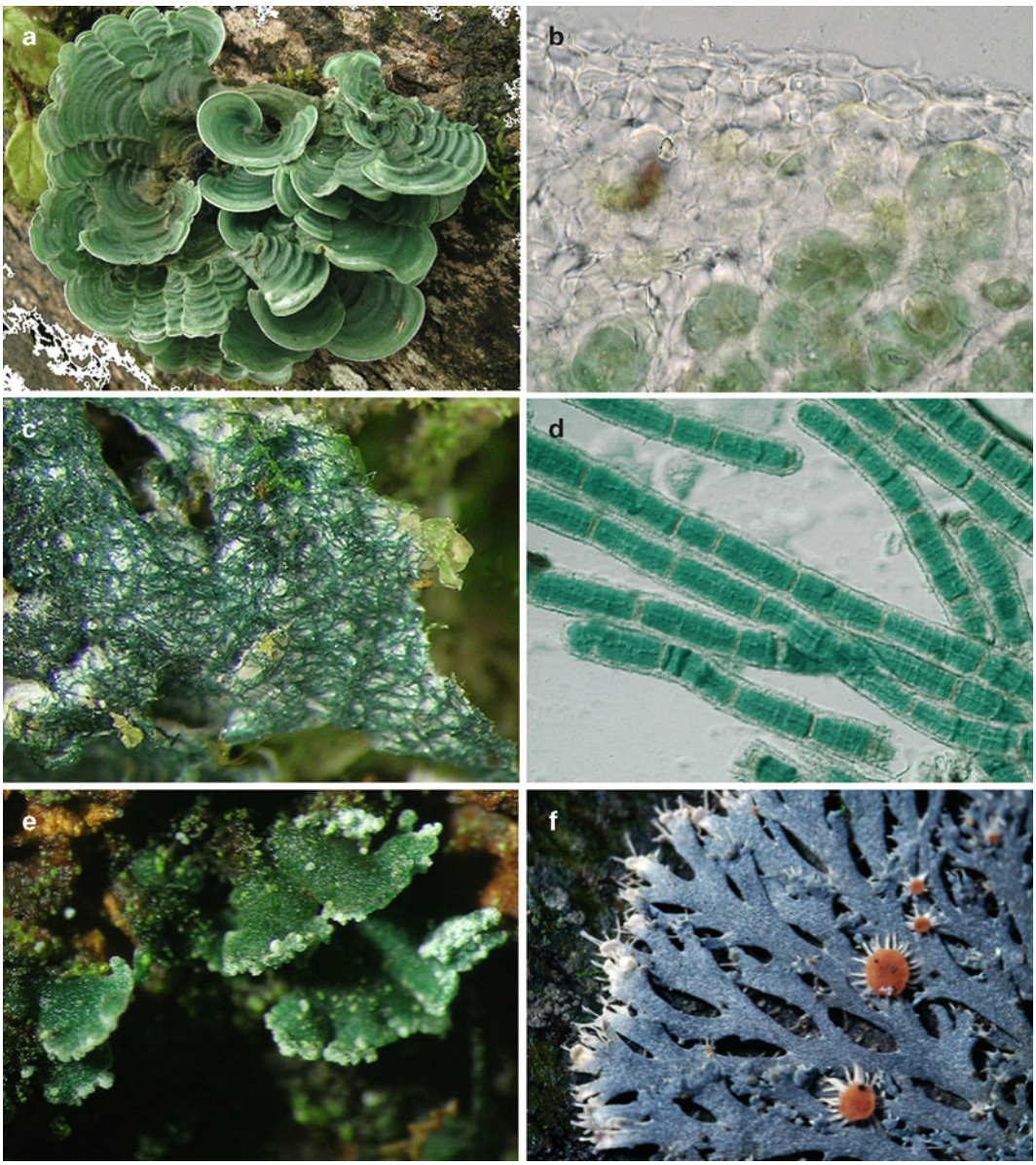

Fig. 23.9 Examples of tropical basidio- and ascolichens associated with a novel lineage of cyanobacterial photobionts. (**a**, **b**) *Dictyonema glabratum* foliose-lobate lichen thallus (**a**) and section through thallus showing cortex and globose photobiont cells (**b**). (**c**, **d**) *Dictyonema schenkianum* appressed-filamentous lichen thallus (**c**) and microscopic view of photobiont filaments surrounded by mycobiont hyphae (**d**). (**e**) *Acantholichen pannarioides* squamulose lichen thallus. (**f**) *Coccocarpia stellata* foliose lichen thallus. (Reproduced with permission from Lücking et al. 2009)

been found in 400 million year old rocks and in much younger amber (Rikkinen and Poinar 2002, 2008; Taylor and Krings 2005). The fossil lichen *Winfrenatia reticulata*, found in 400-million year old Devonian Rhynie chert in Scotland, is thought to have been a primitive form, consisting of a mycobiont and two cyanobionts, which has no analogue in extant lichens (Karatygin et al. 2009). The thallus consists primarily of dead and living filamentous cyanobacteria, implying that the fungus parasitized a cyanobacterial mat. It is clear from molecular studies that lichen symbioses have evolved independently on many occasions and some presently non-lichen-forming fungi may have evolved from ancient lichen-forming ancestors (Lutzoni et al. 2001; Rikkinen 2002).

Desiccation Tolerance

Many lichens experience a daily cycle of drying and wetting, and whilst in the desiccated state their photosynthetic apparatus is protected from potentially damaging levels of radiation both by a sunshade effect resulting from structural changes in the thallus as it dehydrates and by the production of a fluorescence quencher (MacKenzie and Campbell 2001;

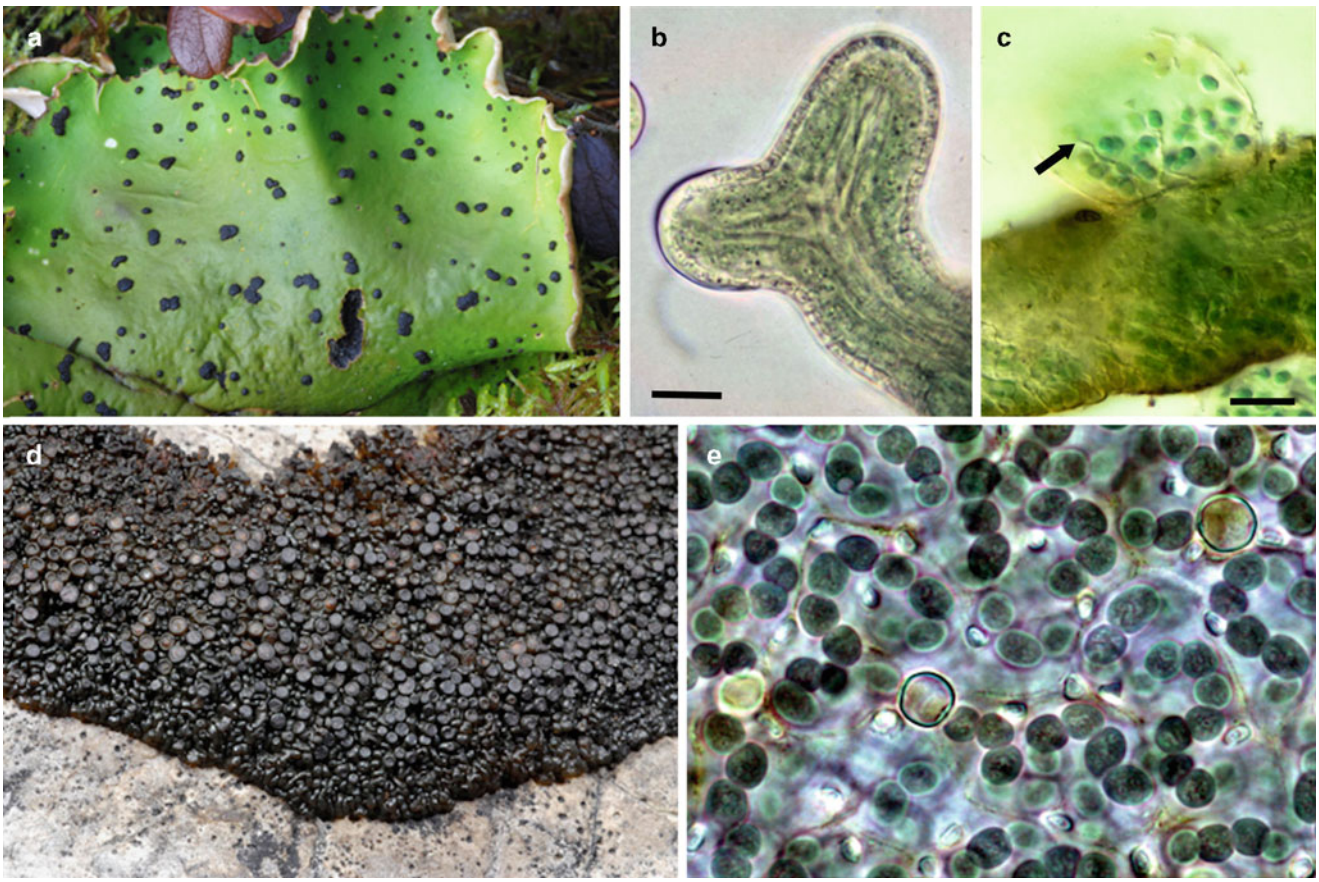

Fig. 23.10 Cyanolichens. (**a**) The tripartite cyanolichen *Peltigera aphthosa*. The green algal photobiont is visible over most of the thallus, whereas the cyanobiont is found in the brown cephalodia scattered across the surface. The white underlying fungal layer can be seen in places around the thallus periphery. (**b**) Lichen *Polychidium* sp. consisting of a single layer of fungal cells forming the cortex which surrounds the cyanobiont, *Scytonema*. (**c**) In the small, fruticose lichen *Lichinella stipatula* branching of the thallus first involves lateral emergence of the cyanobacterial symbiont (probably *Chroococcidiopsis* or *Myxosarcina*), as can be seen in this micrograph. Fungal hyphae (one of which is indicated by the arrow) then grow into the sheath material of the cyanobiont. (**d**) Thallus of the jelly lichen *Collema polycarpon* which has a brown-pigmented *Nostoc* cyanobiont. (**e**) The *Nostoc* cyanobiont of the lichen *Leptogium azureum* photographed through the upper cortex of the thallus. Large, thick-walled heterocysts can be seen amongst the more darkly pigmented vegetative cells. Bars 20 μm (**b**), 80 μm (**c**). (**b**) and (**c**) Reproduced with permission from Sanders WB (2006); (**a**), (**d**) and (**e**) courtesy of Jouko Rikkinen, University of Helsinki

Veerman et al. 2007). Recovery of photosynthesis following dehydration can be rapid in both cyanobacterial and green algal photobionts, although the former always require the presence of liquid water for recovery, whereas the latter can show significant recovery with elevated humidity alone (Palmqvist 2000; Kappen 2000; Lange et al. 2001). This difference is apparent in a lichen photosymbiodeme thallus (Sect. 23.5.5) in which the green algal section recovers photosynthetic activity at high humidity, whereas the cyanobacterial section only does so after rainfall (Schlensog et al. 2000; Green et al. 2002). The domination of green algal over cyanobacterial lichens in habitats such as humid, temperate, evergreen rainforests in New Zealand, northwest United States and Chile may therefore be a consequence of the frequent reactivation of photosynthesis in algal symbionts by humidity alone (Lange et al. 2001). By contrast cyanolichen photosynthesis is often severely limited by water availability. For example, in the lower montane tropical rain forests of Panama, where the tripartite cyanolichens *Lobaria crenulata*, *L. dissecta* and *Pseudocyphellaria aurata* and the bipartite cyanolichens *P. intricata*, *Stricta sublimbata* and *S. weigelii* are highly abundant, the water content of thalli is most favourable for photosynthetic activity at times of low light which limits such activity (Lange et al. 2004). Indeed, at optimum light intensities around noon, thalli are dry and so photosynthesis ceases. Cyanolichens are therefore favoured in moister woodland, or in microhabitats where moss cover or older tree bark provide a wetter environment (Ellis and Coppins 2006).

Desiccation also affects lichen N_2 fixation; in general, the longer the period of desiccation, the longer it takes for full recovery of N_2 fixation, although recovery is rapid in some lichens (Kranner et al. 2008). Another environmental factor that might influence cyanolichen N_2 fixation, particularly in polar regions, is increased UV radiation resulting from ozone depletion (Björn 2007). Indeed, field studies at a subarctic site on Svalbard have shown a 50% reduction in N_2 fixation

by the cyanolichen *Peltigera aphthosa* following 8 years of exposure to artificially-elevated UV-B radiation (Solheim et al. 2002). This appears to be a long-term effect as no reduction was seen after 11 weeks of exposure. *P. aphthosa* has a green algal primary photobiont and the cyanobacteria are found in external cephalodia on the surface of the thallus, fully exposed to UV-B radiation. By contrast, the cyanobacteria in the bipartite lichen *Peltigera didactyla* are protected by the overlying cortex and this may explain why, in similar field experiments, enhanced UV-B radiation had no effect on N_2 fixation in this cyanolichen (Bjerke et al. 2003b).

Habitats

Although a few cyanolichens live in marine littoral waters (Carpenter and Foster 2002), lichens are typically found in most terrestrial ecosystems and can become dominant in areas where their capacity to survive extremes of temperature and desiccation, and their ability to scavenge N or in the case of the cyanolichens fix their own N_2 gives them an advantage over vascular plants (Kappen 2000; Palmqvist 2002; Kranner et al. 2008). Although lichens grow very slowly, some cyanolichens can double their biomass in a year and can make significant contributions to the N budget of specific ecosystems such as montane forests (Büdel et al. 2000; Brown and Dalton 2002; Matzek and Vitousek 2003; Antoine 2004; Campbell and Fredeen 2007; Cusack et al. 2009; Menge and Hedin 2009) and biological soil crusts in arid regions such as the Colorado Plateau of North America (Belnap 2001, 2002) and southwestern Africa (Büdel et al. 2009). In the Colorado Plateau and other dryland regions cyanolichens such as *Collema tenax* and *Collema coccophorum* can be of major importance as part of biological soil crusts, contributing to erosion resistance and to regeneration following ecosystem damage (Bowker et al. 2010). Cyanolichens can also be important colonisers of bare rock such as recent lava flows (Crews et al. 2001; Kurina and Vitousek 2001).

In Antarctica cyanolichens are limited to the maritime regions, possibly because of the availability of the liquid water (from rainfall and meltwater) that they need for recovery of photosynthesis following desiccation (Kappen 2000). In arctic and subarctic regions N inputs from atmospheric deposition are low and the contribution of cyanolichens to the local N economy is significant; this contribution becomes even more important in the more extreme regions where N_2 fixing plants are rare (Weiss et al. 2005; Hobara et al. 2006). Indeed, if vascular plant abundance increases due to global warming this may result in macrolichen decline, as a result of the increased shading by the taller vascular plants and the litter they produce (Cornelissen et al. 2001; Weiss et al. 2005). In Arctic tundra, N_2 fixing lichens such as *Peltigera aphthosa* and *P. polydactyla* seem to be limited by phosphorus availability, because P-fertilisation can stimulate N_2 fixation and increase lichen nitrogen concentration (Weiss et al. 2005). By contrast, lichen abundance decreases significantly with ammonium nitrate fertilisation, perhaps as a result of increased shading by vascular plants (Weiss et al. 2005).

Cyanolichens are a particularly important part of the epiphyte community in the forests of the northern hemisphere, their prevalence increasing with the age of a forest and indeed, they are often restricted to old-growth forests (Sillett et al. 2000; Peterson and McCune 2001; Hedenås and Ericson 2004) although there are exceptions (Peterson and McCune 2003; Menge and Hedin 2009). Limitations in dispersal ability and diaspore production may be factors that restrict cyanolichens largely to old-growth forests (Hilmo 2002). Whereas lichens with green algal symbionts are ubiquitous, cyanolichens prevail in shady, humid stands in a boreal forest landscape (Hedenås and Ericson 2004), or in microhabitats where moss cover or older tree bark provide wetter conditions (Ellis and Coppins 2006). In addition, in these same forests the occurrence of cyanolichens correlates with the occurrence of their free-living cyanobionts, particularly on the shady, northern side of the tree trunks (Hedenås et al. 2007). Similarly, cyanolichen species richness and biomass are greatest in the more shady and humid parts of montane rainforests in Panama, where almost half of all lichen species are cyanolichens (Büdel et al. 2000).

In general, the factors that influence the occurrence and diversity of cyanolichens in a forest are, in decreasing order of importance, air quality, climate, elevation, soil nutrient status, forest age, proximity to deciduous trees, soil moisture and stand spacing (Goward and Arsenault 2000a, b). In some montane forests phosphate availability may constrain cyanolichen abundance as P-fertilisation can result in stimulation of the whole epiphyte community, but especially the cyanolichens (Benner and Vitousek 2007; Benner et al. 2007; McCune and Caldwell 2009). Where acid rain is prevalent cyanolichens are restricted to well-buffered bark, such as that of *Fraxinus* (ash), and can be lost from trees, such as *Quercus* (oak) and conifers, with more acidic bark (Richardson and Cameron 2004). This is a problem in much of Europe, but not in the west of Canada, nor in the American Pacific Northwest (Goward and Arsenault 2000a, b). In young forests of humid south-central British Columbia epiphytic cyanolichens, including the tripartite *Lobaria pulmonaria*, grow on the lower branches of conifers where calcium-rich leachates from adjacent *Populus* trees help to increase the pH of the conifer bark and encourage the initial establishment of epiphytic cyanolichens, although once they are established the presence of *Populus* is no longer essential (Goward and Arsenault 2000a). In forests on the border of Idaho cyanolichens only occur on the conifer branches that have low Mn/Ca and Mn/Mg ratios, which occurs within the drip zones of *Populus* trees (Hauck and Spribille 2002), implying that the ratio of minerals is more important than their concentration.

The tripartite cyanolichen *Lobaria pulmonaria* is still widespread in the northern hemisphere, but its abundance has decreased due to air pollution and forest management practises

(Gu et al. 2001). Factors affecting the spread of this lichen are the availability of suitable trees (notably aspen and willow) and the proximity of lichen-occupied trees (Gu et al. 2001). Although dispersal capacity may be a factor in limiting *L. pulmonaria* distribution (Öckinger et al. 2005), some consider ecological factors more important (Werth et al. 2006). Transplantation experiments have also shown that *L. pulmonaria* seldom achieves its growth potential in its natural ecological niches where there is a trade-off between optimum light intensity and the risk of desiccation damage, both of which occur higher in the tree canopy (Gauslaa et al. 2006). In deciduous forests cyanolichens such as *Lobaria pulmonaria* have to adapt to large fluctuations in light availability from low light in the summer when they are shaded by the tree canopy, to much higher light levels in winter and spring (MacKenzie et al. 2001). The best times for growth are the transition periods in spring and autumn when temperatures are relatively high, but the light is also at its greatest without the tree canopy. Both the intensity and the spectral quality of light available to lichens are also affected by the tree species and this in turn can result in chromatic adaptation in lichen cyanobionts and photobionts (Czeczuga et al. 2006, 2010).

Cyanolichens form a relatively small proportion of total forest biomass, but their abundance and N-rich thalli mean that they make substantial contributions to forest N particularly in old-growth forests which are commonly N-deficient (Campbell and Fredeen 2007; Botting et al. 2008). Nitrogen is released either by leaching from the lichen thallus or by decomposition of lichen litter (Holub and Lajtha 2003; Caldiz et al. 2007; Cornelissen et al. 2007). The thalli of bipartite cyanolichens generally have the highest N content, tripartite lichens lower and bipartite chlorolichens the lowest (Palmqvist et al. 2002; Caldiz et al. 2007; Botting et al. 2008). In addition, bipartite cyanolichens often show the most rapid rates of decomposition (Caldiz et al. 2007).

In forests, atmospheric deposition and litter decomposition can be the major contributors to the N budget, but in some northern forests, such as those in the American Pacific Northwest, atmospheric deposition is much less and the cooler temperatures, combined with the mainly lignin-rich coniferous litter, limit the rate of decomposition of soil organic matter, with the result that the contribution of cyanolichens is of greater significance, especially in old-growth or late-successional forests (Holub and Lajtha 2004; Knowles et al. 2006). For example, increases in soil N content can be measured up to 1.5 m away from thalli of terricolous cyanolichens of the genus *Peltigera* (Knowles et al. 2006) and epiphytic cyanolichens such as *Lobaria* can contribute 2.5–4.5 kg N ha^{-1} $year^{-1}$, which represents 33–67% of new N inputs into the local ecosystem (Holub and Lajtha 2004).

In addition to N_2 fixed by their cyanobionts, cyanolichens can obtain inorganic nitrogen such as nitrate and ammonium, and organic forms such as amino acids, from rainwater and canopy through-fall. Indeed, these forms of N are taken up by cyanolichens, although there seems to be little correlation between rates of uptake and lichen morphology or microhabitat (Dahlman et al. 2004). Despite large variations in N supply cyanolichens are able to maintain a steady thallus N content and to regulate the distribution of nitrogen and carbon resources around the thallus (Sundberg et al. 2001; Dahlman et al. 2002, 2004; Kytöviita and Crittenden 2007).

In addition to the more obvious environmental influences described above, forest management practices can have significant effects on cyanolichen survival and abundance. With the fragmented nature of much woodland in Europe, existing patches of old-growth woods may be vital for the preservation of lichens in times of environmental stress such as global warming (Ellis et al. 2009). The aspen (*Populus tremula*) is a common host of epiphytic cyanolichens in Sweden, but new stands of aspen are often not colonised for 50 or more years, so loss of trees or changes to the forest structure or composition, can lead to a long-term decline in cyanolichen abundance (Hedenås and Ericson 2004), and careful management of forests is vital for the preservation of lichen populations (Hedenås and Ericson 2003; Richardson and Cameron 2004; Hedenås and Hedström 2007). Cyanolichens are most common on aspen stands within coniferous forests, and are rare on aspen growing in previously agricultural land, possibly because they are at an advantage in the forest where N availability is low (Hedenås and Ericson 2004). However, not all cyanolichens respond in the same way to changes in forestry practices. For example, of three foliose cyanolichens in the Collemataceae, *Collema curtisporum* and *C. furfuraceum* are 5–6 times less frequent in aspen-poor coniferous forest than in aspen-rich coniferous forest, whereas *Leptogium saturninum* is unaffected by aspen abundance, perhaps because it has better dispersal abilities (Hedenås and Ericson 2008). However, even following dispersal the juvenile stages of epiphytic cyanolichens are sensitive to environmental factors which may result in very low growth and colonization (Hilmo and Ott 2002).

A final major influence on lichen populations is likely to be climate change, but a study by Ellis and Coppins (2007) illustrates the difficulties of predicting the outcomes. They modelled a community of lichen epiphytes, consisting of 80% cyanolichens, and known as the *Lobarion*, named after *Lobaria pulmonaria*. This population, which is characteristic of the cool temperate forests of western Scotland and south-western Norway, is sensitive to climatic and habitat changes and could be favoured by predicted increases in average annual temperatures and winter precipitation, which might result in an increase in its current range (Ellis and Coppins 2007). However, modelling revealed a complex relationship between temperature, precipitation and woodland structure, such that the response of the *Lobarion* to climate change may be modified by the current, rather than the future forest landscape. In a recent study of lichens in Italy, Marini et al. (2011) concluded that the future impacts of climate change

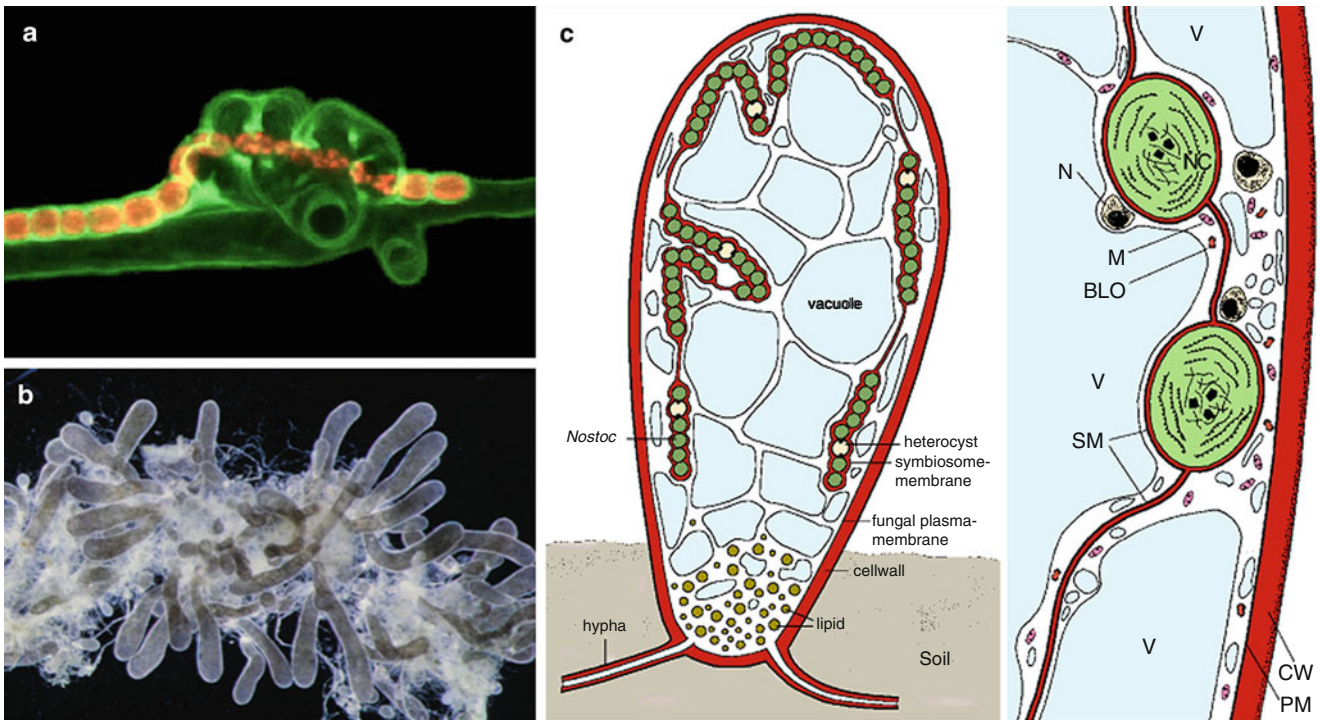

Fig. 23.11 The *Geosiphon-Nostoc* symbiosis. (**a**) Confocal laser scanning microscopic projection of a *Geosiphon* hypha engulfing a *Nostoc* filament (the cells of which are 3–5 μm in width). The fungal cell wall and the *Nostoc* extracellular polysaccharides have been labelled with the fluorescence-coupled lectin ConA (green). In the centre of the image the *Nostoc* cells (red) that have been engulfed show deformations and reduced pigment fluorescence, whereas those on either side retain their normal appearance. (**b**) The fully-developed symbiosis in which the cyanobiont is contained in bladders, the largest of which are about 1.5 mm in length. (**c**) Diagrammatic representation of the *Geosiphon-Nostoc* symbiosis, showing the compartmentation of the cyanobiont. The drawing to the right shows an enlargement of the peripheral region of the bladder. Drawings are based on electron microscope observations. *BLO* bacteria-like organism, *CW* cell wall, *M* mitochondrion, *N* nucleus, *NC Nostoc* cell, *PM* plasma membrane, *SM* symbiosome membrane, *V* vacuole. (Reproduced with permission from Adams et al. 2006)

on lichen species richness is likely to vary with photobiont (green algal or cyanobacterial) type.

Lichens as Food

Lichens form a vital part of the winter diet for reindeer and caribou (den Herder et al. 2003), yet these animals avoid eating cyanolichens, even when starving, despite them being a far richer source of N than green algal lichens (Rai 2002). This was thought to be because of the poor digestibility of some cyanolichen species (Storeheier et al. 2002), but there is an interesting alternative or additional explanation. The *Nostoc* symbionts of the cyanolichens *Pannaria pezizoides* and *Peltigera leucophlebia* have been shown to produce hepatotoxic microcystins, both in the cephalodia (in the case of the latter lichen) and when grown free-living in culture and it might be this cyanobacterial toxin that deters grazers (Oksanen et al. 2004; Kaasalainen et al. 2009). As Kaasalainen et al. (2009) have pointed out, the production of microcystin by lichen-associated *Nostoc* raises concerns about the safety of such lichens used in China as food and in traditional medicine. Although in many tripartite lichens it is the cephalodia that are avoided by grazers, the arctic tripartite cyanolichen *Nephroma arcticum* has a *Nostoc* photobiont in internal cephalodia which are preferentially grazed by slugs, while the green algal parts of the thallus are mostly left alone (Asplund and Gauslaa 2010).

23.2.2.2 *Geosiphon pyriformis*

The *Geosiphon pyriformis-Nostoc* symbiosis (referred to as *Geosiphon pyriforme* in older literature) is the only known example of an endocytobiotic cyanobacterium-fungus association (Kluge et al. 2002; Schüßler 2002; Adams et al. 2006; Bergman et al. 2007a). The fungal host belongs to the arbuscal mycorrhizal (AM) and related fungi within the phylum *Glomeromycota* (Schüßler et al. 2001; Kluge 2002; Adams et al. 2006). The cyanobiont, *Nostoc punctiforme*, is found intracellularly within specialised bladders produced by the fungal hyphae (Fig. 23.11). Although the cyanobacterium can be grown free of the fungus, the fungus appears to be an obligate symbiont (Schüßler 2006). The function of the *Nostoc* seems to be primarily the provision of photosynthate for the fungus, although the presence of heterocysts and high nitrogenase activity within bladders clearly indicate that N_2 fixation also occurs (Adams et al. 2006). The *Nostoc*-containing bladders can only take up molecules with a diameter less than

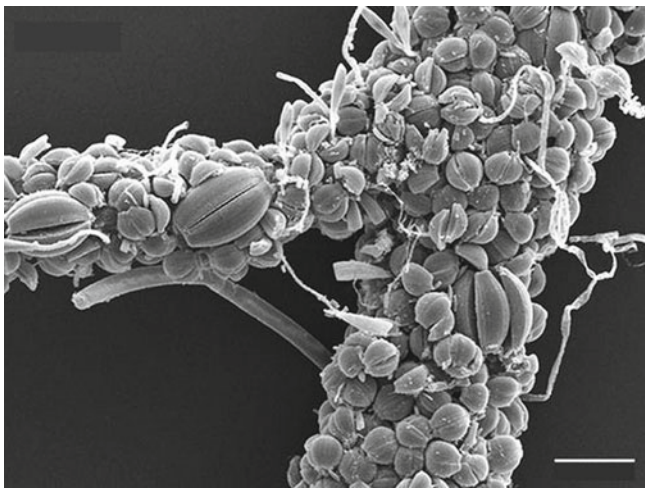

Fig. 23.12 Scanning electron micrograph of the filamentous freshwater green alga *Cladophora* covered with epiphytic diatoms. The larger diatoms are *Epithemia turgida* and the smaller ones are *E. sorex*, both of which contain N_2-fixing cyanobacterial endosymbionts. Bar 50 µm. (Photograph by Rex Lowe. Reproduced with permission from Power et al. 2009)

0.45 nm, which excludes sugars but not inorganic ions such as phosphate which can therefore be supplied to the cyanobiont. Indeed, phosphate limitation is a strong promoter of the formation of the association (Adams et al. 2006) (Fig. 23.12).

The *Geosiphon pyriformis-Nostoc* symbiosis is found in the upper layers and on the surface of moist, nutrient-poor soils, particularly those low in phosphate. It seems to be rare, there having been only five reports of its occurrence in nature, at sites from Eastern Germany to Austria. Although it can be grown successfully in the laboratory it is difficult to obtain large amounts of material for experimentation (Kluge et al. 2002; Adams et al. 2006). In nature the *Nostoc* cyanobiont is thought to be released from decaying *Geosiphon* bladders in the form of akinetes which subsequently germinate. There is evidence that the same *Nostoc* symbionts can be shared by *Geosiphon pyriformis*, the liverwort *Blasia* and the hornwort *Anthoceros*, which are all found in close proximity in their natural environment (Adams et al. 2006).

23.2.3 Diatoms

A number of mostly marine diatom-cyanobacteria symbioses are known and there are surely more to be discovered, particularly as the cyanobiont is often difficult to visualise by microscopy. The heterocystous cyanobacterium *Richelia intracellularis* (Hindák 2000), which consists of a short filament of 3–10 vegetative cells with a single heterocyst at one end, is found as an endosymbiont in diatoms of genera *Rhizosolenia* and *Hemiaulus* which are abundant in tropical and sub-tropical seas (Fig. 23.13c; Bergman 2001; Janson 2002; Carpenter 2002; White et al. 2007; Bar Zeev et al. 2008; Foster et al. 2007, 2009; Wouters et al. 2009; Bombar et al. 2011). *R. intracellularis* has also been found as an epiphyte on the diatom *Chaetoceros compressus* (Fig. 23.13a, b) in the Pacific and Indian Oceans (Gómez et al. 2005) and in the Atlantic Ocean (Foster et al. 2009). The epiphyte of *Chaetoceros* has sometimes been referred to as *Calothrix rhizosoleniae*, although Foster and Zehr (2006) have shown it to be closely related to, but distinct from, the *Richelia* endophytes from *Hemiaulus hauckii* from the North Atlantic and *Rhizosolenia clevei* from the North Pacific. These N_2 fixing *Richelia*-diatom symbioses may make major contributions to the N budgets of the areas of ocean where they are abundant, especially when they form blooms, some of which can cover areas of over 100,000 km^2 (Zehr et al. 2000; Arrigo 2005; Mahaffey et al. 2005; White et al. 2007; Foster et al. 2009). Foster et al. (2011) have recently demonstrated the mutualistic nature of these diatom-cyanobacteria symbioses. *Richelia* was show to fix far more N_2 than was needed for its own use, up to 97.3% of this N being transferred to the host. In turn, rates of both N_2 fixation and growth of the *Richelia* symbionts were much greater in symbiosis than in the free-living state.

Unicellular cyanobacteria are also found as symbionts of diatoms. For example, the chain-forming diatoms *Neostreptotheca* and *Streptotheca*, which are common in the tropics, house numerous 3–5 µm diameter cyanobacterial cells in their cytoplasm (Carpenter 2002). Each cell of another chain-forming diatom, *Climacodium frauenfeldianum*, contains 20–30 coccoid cyanobacteria, thought to be related to the N_2 fixing genus *Cyanothece* (Carpenter and Janson 2000; Carpenter 2002). The cytoplasm of diatoms *Rhopalodia gibba* and *Epithemia turgida* contains two to five unicellular endosymbionts known as spheroid bodies, which are clearly related to cyanobacteria yet appear to have lost the capacity for photosynthesis (Janson 2002; Prechtl et al. 2004; Kneip et al. 2008; Bothe et al. 2010). The *Rhopalodia/Epithemia*-cyanobacteria associations can fix N_2 in the light and phylogenetic analysis of cyanobiont 16S rDNA and *nifD* has revealed a close relationship to the N_2 fixing cyanobacterium *Cyanothece* (Prechtl et al. 2004; Bothe et al. 2010).

A freshwater example of a cyanobacterial-diatom symbiosis is found in the Eel River in California, U. S. A., where heavy growths of the macroalga *Cladophora* become overgrown by the diatoms *Epithemia turgida* and *Epithemia sorex*, and to a lesser extent *Rhopalodia gibba*, all with cyanobacterial endosymbionts (Fig. 23.12; Power et al. 2009). These epiphytic *Epithemia* biofilms can fix N_2 at rates ranging from 0.3 to 1.7 µg N g^{-1} (dry wt) h^{-1}. The great abundance of *Cladophora* increases the functional surface area of the littoral zone by a factor of up to 2×10^5 and when this surface is covered by N_2 fixing *Epithemia*, the contribution to ecosystem N could be enormous (Power et al. 2009).

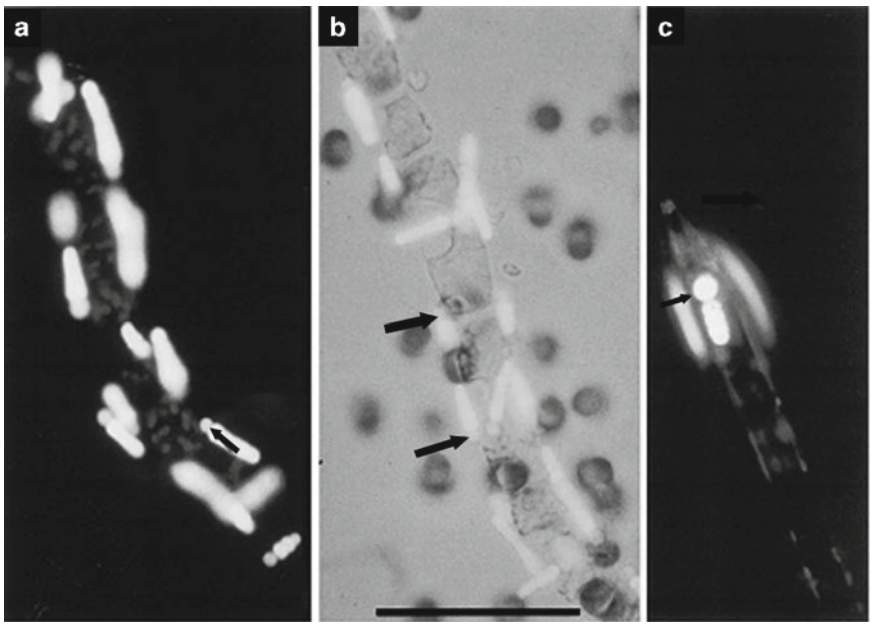

Fig. 23.13 Diatom symbioses with the cyanobacterium *Richelia intracellularis*. (**a**) Epifluorescence micrograph of fluorescing *R. intracellularis* filaments attached to the outside of the chain-forming diatom *Chaetoceros* with its more weakly fluorescing chloroplasts. The arrow indicates the single small heterocyst found at one end of each filament. (**b**) Merged fluorescence and incident light images of *Chaetoceros* with epibiotic *R. intracelluaris* filaments. The dark, roughly circular patches in the background are the pores of the filter used to concentrate the sample. (**c**) Single *R. intracellularis* filament inside *Rhizosolenia clevei* var. *communis*. The arrow indicates the single large heterocyst. Bar 50 μm. (Reproduced with permission from Janson et al. 1999)

A three-membered symbiosis is formed by the centric diatom *Leptocylindrus mediterraneus* which carries in its girdle bands the aplastidic protist *Solenicola setigera*, found as groups of cells together with abundant coccoid cyanobacteria, thought to be *Synechococcus*, embedded in the protist's extracellular matrix (Carpenter 2002). Although both the protist and the *Synechococcus* are widely-distributed, the three-membered symbiosis seems to be rare.

A potentially symbiotic relationship is formed by motile diatoms of the genera *Amphora*, *Berkeleya*, *Cymbella*, *Entomoneis*, *Epithemia*, *Lunella*, *Mastogloia*, *Nitzschia* and *Rhopalodia* which are found within colonies of the heterocystous cyanobacterium *Rivularia* Roth in the Baltic Sea (Snoeijs and Murasi 2004). This is thought to benefit the diatoms by protecting them from grazing and physical disturbance, providing mucilage as a substratum for motility and supplying nutrients released by the *Rivularia*, although the benefits to the cyanobacteria are unclear.

Another unusual interaction between cyanobacteria and diatoms is the formation of "microbial spheres" found in North Sea microbial mats (Brehm et al. 2003). These spheres are up to 3 mm in diameter and consist of a complex community of heterotrophic bacteria, diatoms (*Navicula perminuta*) and cyanobacteria (*Phormidium*) embedded in extracellular polymeric substances and surrounded by a membrane of unknown composition. The spheres can be maintained in laboratory culture for 3 years or more and can become calcified, producing ooids (Brehm et al. 2006). Similar microbial consortia have been found in a desert spring in Mexico (Garcia-Pichel et al. 2002). These 'waterwarts' are roughly spheroid or elongated colonies approximately 1 cm in diameter, consisting of *Aphanothece*-like unicellular cyanobacteria embedded in large amounts of gel-like glycan. These colonies support an assemblage of filamentous cyanobacteria (including possible *Phormidium*, *Pseudanabaena* and *Lyngbya*) and diatoms (primarily *Nitzschia*). The colonies also invariably contain crystals of calcite which are thought to act as ballast, preventing the colonies from being washed out of the spring by the upwelling water (Garcia-Pichel et al. 2002).

23.2.4 Dinoflagellates, Radiolarians, Tintinnids, Euglenoids and Foramenifera

Non-photosynthetic dinoflagellates (Dinophycaea) were shown to harbour epi- or endobiotic "phaeosomes" over a century ago (see: Carpenter 2002; Carpenter and Foster 2002; Foster et al. 2006a). These phaeosomes are now known to be symbiotic cyanobacteria, but for a long time little was known about them. Members of six dinoflagellate genera, *Ornithocercus*, *Histioneis*, *Parahistioneis*, *Citharistes*, *Dinophysis* and *Amphisolenia*, have been reported to contain a range of unicellular symbiotic cyanobacteria (Fig. 23.14; Carpenter 2002;

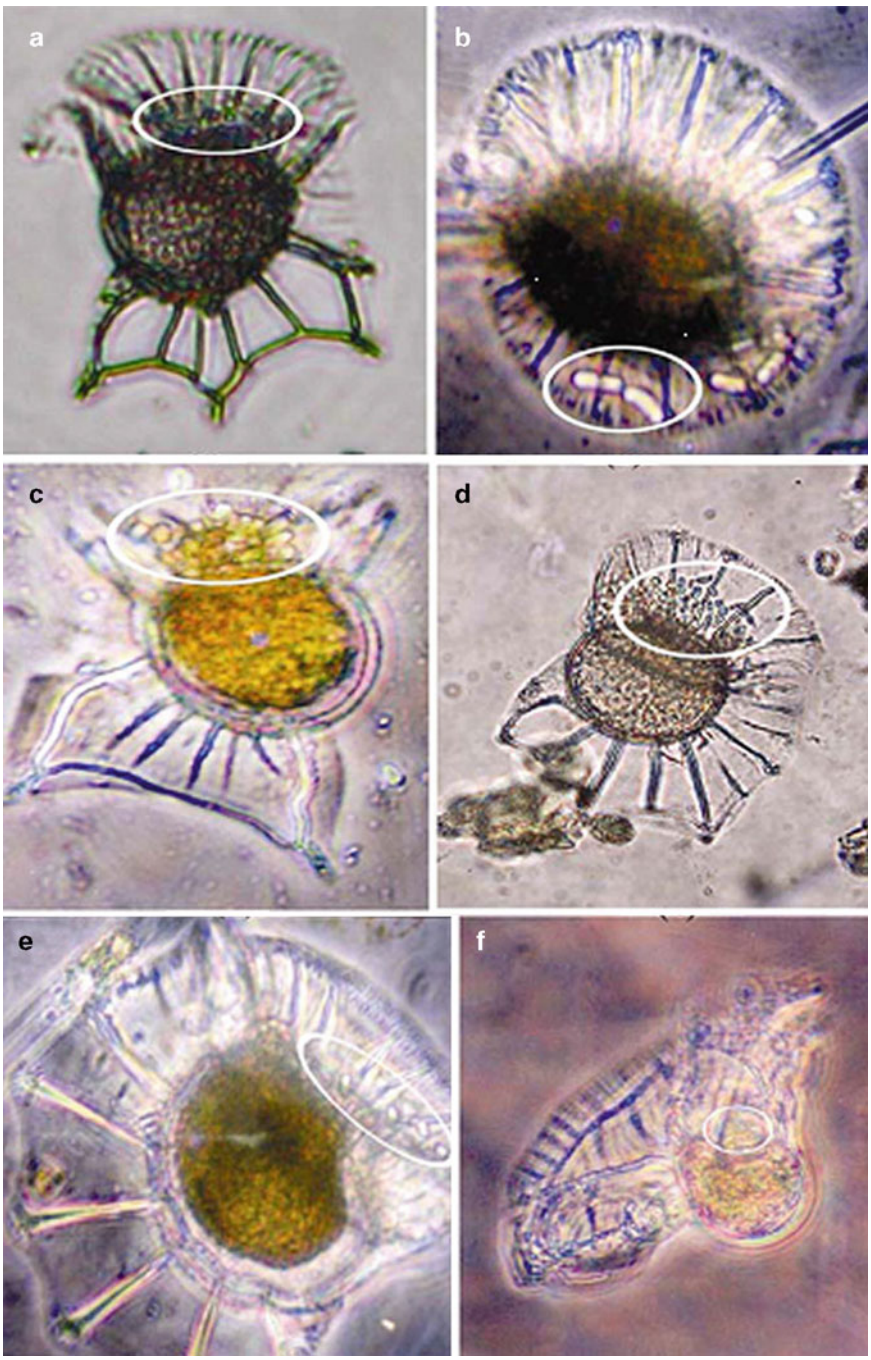

Fig. 23.14 Dinoflagellate-cyanobacteria symbioses. Heterotrophic dinoflagellates (**a**) *Ornithocercus magnificus*, (**b**) *O. quadratus*, (**c**) *O. heteroporus*, (**d**) *O. thumii*, (**e**) ***O****. steinii* and (**f**) *Histioneis hyaline* (**f**) with the location of the cyanobionts indicated by circles. (Reproduced with permission from Jyothibabu et al. 2006)

Jyothibabu et al. 2006; Tarangkoon et al. 2010), which in the case of *Histioneis* label positively with anti-nitrogenase antibodies, so may be capable of N_2 fixation (Foster et al. 2006a).

The radiolarians are amoeboid protozoa with mineral skeletons and at least two of them, *Spongostaurus* and *Dictyocoryne truncatum*, host symbionts thought to be related to *Prochlorococcus* (Foster et al. 2006a, b). Tintinnids are conical or trumpet-shaped protozoan ciliates, at least one of which, the open-ocean tintinnid *Codonella*, hosts cyanobacterial symbionts (Carpenter and Foster 2002; Foster et al. 2006a). An apparently transient and rare endosymbiosis is formed between the euglenoid *Petalomonas sphagnophila* and *Synechocystis*-like unicellular cyanobacteria (Schnepf et al. 2002). This apoplastidic euglenoid flagellate is found in floating mats of *Sphagnum* moss in bog lakes in Germany and the cyanobacteria were once thought to be food particles, but they remain alive for several weeks or longer inside a perialgal vacuole, even though digestion of food particles is usually complete within a few hours. The foraminifera (amoeboid protists) *Marginopora vertebralis* and *Amphisorus*

hemprichii, have also been reported to contain endophytic cyanobacteria (Lee 2006).

23.2.5 Animals

23.2.5.1 Sponges

Sponges (phylum *Porifera*) are some of the most ancient metazoan animals, with a fossil record dating back over 580 million years to the Precambrian (Taylor et al. 2007). Research on sponges has increased rapidly in the last decade or so, partly because of the interest in the complex populations of symbiotic microorganisms they host, but perhaps largely because they produce a wide array of biologically-active secondary metabolites, some of which may be produced by their symbionts, which include cyanobacteria (Lee et al. 2001; Flatt et al. 2005; Ridley et al. 2005a, b; Schmidt et al. 2005; Taylor et al. 2007; Kennedy et al. 2007, 2008; Simmons et al. 2008; Selvin et al. 2010; Sacristan-Soriano et al. 2011; Li et al. 2011). The symbiotic microorganisms in sponges can constitute up to 40% of the host biomass and can exceed the microbial concentration in the surrounding seawater by up to four orders of magnitude (Friedrich et al. 2001; Webster and Hill 2001; Hentschel et al. 2006; Taylor et al. 2007; Schmitt et al. 2008). The sponge microbial population can be highly diverse; for example, from 10 individuals of the sponge *Candidaspongia flabellate* Burja and Hill (2001) were able to isolate in culture 228 different bacterial species, 25 fungi, 3 actinomycetes and 9 cyanobacterial strains. In photosynthetic sponges the symbionts include eukaryotic rhodophytes, diatoms, dinoflagellates and chlorophytes, but the most important and abundant group is probably the cyanobacteria (Wulff 2006; Hentschel et al. 2006; Taylor et al. 2007; Usher 2008; Hardoim et al. 2009).

Of the sponges hosting cyanobacterial symbionts (cyanosponges) 100 species are known (Diaz et al. 2007; Usher 2008) and they typically constitute 30–50%, but sometimes up to 90%, of the sponges on tropical reefs (Usher 2008). They are thought to be the most ancient of the microorganism-metazoan interactions (Hentschel et al. 2006). Cyanosponges were once thought to be mostly restricted to the nutrient-poor water of tropical regions, but later reports suggest they are just as common in temperate waters (Usher 2008). Their colours usually result from the cyanobiont phycobiliproteins, the ratios of which can vary depending on the amount of light received, resulting in colour changes from yellow/green in high light to red/brown in low light (Usher et al. 2004a). Cyanobacterial pigments may also provide the sponge with protection from excessive sunlight, particularly in intertidal zones (Taylor et al. 2007). Sponge cyanobionts seem to substantially enhance host growth rates in at least two Caribbean coral reef sponges, *Aplysina fulva* and *Neopetrosia subtriangularis* (Erwin and Thacker 2008a). Indeed, cyanosponges in general seem to be faster growing and more competitive for space than the non-photosynthetic sponges and can even overgrow and kill live coral (Diaz et al. 2007; Usher 2008; Tang et al. 2011; Hirose and Murakami 2011). However, the degree of dependence in cyanobacteria-sponge associations may vary. For example, when artificially shaded the marine cyanosponges *Lamellodysidea chlorea* and *Xestospongia exigua* respond differently, the former losing mass while its cyanobiont (the filamentous *Oscillatoria spongeliae*) doesn't change in abundance, implying a mutualistic relationship, whereas the latter does not lose mass but its cyanobiont (the unicellular *Synechococcus spongiarum*) decreases in abundance, implying a commensal relationship (Thacker 2005). Similarly, bleaching of the *Synechococcus* symbionts of the giant barrel sponge *Xestospongia muta* in the Florida Keys does not result in sponge mortality (McMurray et al. 2011). By contrast, bleaching of the Mediterranean sponge *Ircinia fasciculata* results in the death of its symbiotic cyanobacteria and the subsequent death of the sponge itself (Cebrian et al. 2011).

Cyanosponges play important roles in reef ecology as nutrient cyclers and primary producers and they provide food and a habitat for a wide range of organisms (Usher 2008). Their cyanobacterial symbionts confer several advantages over the zooxanthellae in other photosynthetic sponges, because they have a wider temperature tolerance, produce sunscreens and can photosynthesise at very low light, enabling their hosts to grow in full sun in intertidal zones and at low light in shaded areas and even in caves (Usher 2008). Although digestion of the cyanobionts, as a potential food source, was reported in earlier studies, it is likely that this is rare and may be a result of poor health of one of the partners (Usher 2008). Cyanobiont secondary metabolites can provide the host with protection from grazing, although some cyanobacteria may actually attract predators to feed on sponges, as the mollusc *Tylodina perversa* chooses to feed on areas of *Aplysina aerophoba* rich in symbiotic cyanobacteria, but it shows no interest in the closely-related *Aplysina cavernicola* which lacks cyanobionts (Becerro et al. 2003).

23.2.5.2 Corals

The primary photosynthetic symbionts of scleractinian (stony) corals are a diverse group of endosymbiotic dinoflagellates (zooxanthellae) of the genus *Symbiodinium* which are found within the gastrodermal cells of the host (Knowlton and Rohwer 2003; Rosenberg et al. 2007; Chen et al. 2011). However, corals also harbour diverse bacterial communities (Rosenberg et al. 2007; Chen et al. 2011) and cyanobacterial symbionts are found in at least one coral, *Montastreae cavernosa* (Lesser et al. 2004, 2007). Cyanobacterial sequences have also been found by PCR amplification of total DNA from three coral species, *Montastraea franksi*, *Diploria strigosa* and *Porites astreoides* using bacteria-specific 16S rDNA primers (Rohwer et al. 2001, 2002). In addition, cyanobacterial DNA sequences have been identified in the metagenome

Fig. 23.15 Caribbean scleractinian (stony) coral *Montastraea cavernosa* showing orange daytime fluorescence from the phycoerythrin of its unicellular cyanobacterial endosymbionts. The N_2 fixing intracellular cyanobionts co-exist with zooxanthellae. The colony is approximately 0.6 m in height. (Reproduced with permission from Lesser et al. 2004)

of the bacterial community from the coral *Porites compressa* (Thurber et al. 2009) and the cyanobacterial *nifH* gene has been detected in *Montipora* spp. (Olson et al. 2009).

The characteristic sun-induced orange-red fluorescence of the Caribbean coral *Montastreae cavernosa* (Fig. 23.15) is derived from the red cyanobacterial photopigment phycoerythrin, found in 1.0–3.0 μm diameter unicellular cyanobacteria within the epithelial cells of the host and surrounded by host cell membrane (Lesser et al. 2004). The 16S ribosomal DNA sequence of these coccoid cyanobacteria is most closely related to either *Synechococcus* or *Prochlorococcus* in the order Chroococcales. The fluorescence is apparently caused by detachment of the phycoerythrin from the photosynthetic apparatus, induced by the presence of high concentrations of glycerol, which is the major carbon compound transferred from the zooxanthellae to the coral host and which may also act as a carbon source for the cyanobionts (Lesser et al. 2004). The cyanobacteria are capable of N_2 fixation, which is confined to the night when the host tissues revert from their daytime hyperoxia to the hypoxia or anoxia that favours N_2 fixation (Lesser et al. 2007). At least some of the N_2 fixed is found in the zooxanthellae. If N_2-fixing cyanobacteria prove to be widespread in corals, they (together with the many other N_2-fixing bacteria) may play a significant role in the N-budget of coral reefs (Lesser et al. 2007).

The calcareous skeleton of scleractinian corals can provide a home for endolithic cyanobacteria and algae. For example, the encrusting coral *Oculina patagonica*, with its endosymbiotic zooxanthellae harbours endolithic chlorophytes of the genus *Ostreobium* in its skeleton (Fine and Loya 2002). Transfer of the products of photosynthesis from the endolithic algae to the coral may help survival of the coral during periods of bleaching when loss of the symbiotic zooxanthellae allows more light to reach the endoliths (Fine and Loya 2002). The endolithic, filamentous cyanobacterium *Plectonema terebrans* can be found burrowing into the calcareous skeleton of the cold-water corals *Desmophyllum dianthus* and *Caryophyllia huinayensis*, which lack zooxanthellae (Försterra and Häussermann 2008). The cyanobacterium is visible as a pink to violet discolouration of the corallite (Fig. 23.16) and is frequently found together with the endolithic filamentous green alga *Ostreobium queckettii* (Fig. 23.16), with the cyanobacterium generally most abundant on the light-facing side of the corallite. Both endoliths are most abundant where the corallite is covered with polyp tissue, possibly as a result of protection from grazers. Excreted metabolites of the endolithic phototrophs may be beneficial to the host polyp although the nature of these metabolites is not known (Försterra and Häussermann 2008).

23.2.5.3 Ascidians

Ascidians or sea squirts are sac-like marine invertebrate filter feeders, approximately 30 of which, from four genera of the Didemnidae (*Didemnum*, *Trididemnum*, *Lissoclinum* and *Diplosoma*), have been reported to form symbioses with cyanobacteria, although many species in each genus are non-symbiotic (Hirose and Hirose 2007). The symbiosis is thought to have arisen independently in the Didemnidae at least once in each genus (Yokobori et al. 2006; Münchhoff et al. 2007). All symbiotic species are colonial forms from sub-tropical or tropical marine waters and in most cases they harbour unicellular cyanobacteria of the genus *Prochloron* (Fig. 23.17), which contain chlorophyll *a* and *b* but lack phycobilin pigments (Griffiths 2006). However, the unicellular cyanobacterium *Synechocystis trididemni*, a close relative of *Prochloron*, is found in *Trididemnum* species (Münchhoff et al. 2007), and *Trididemnum clinides* harbours three different cyanobionts, two unicellular and one filamentous, none of which seem to be *Prochloron* (Hirose et al. 2009b).

The chlorophyll *d*-containing cyanobacterium *Acaryochloris marina* occurs as an epibiont on the undersides of some didemnid ascidians found on the Great Barrier Reef (Fig. 23.17; Kühl et al. 2005; Larkum and Kühl 2005). The ascidian tissue strongly attenuates visible light, but the far-red light absorbed by chlorophyll *d* penetrates easily and so the underside of the animal is an ideal niche for *Acaryochloris*. However, this cyanobacterium may not be restricted to the surface of tunicates as a recent study found

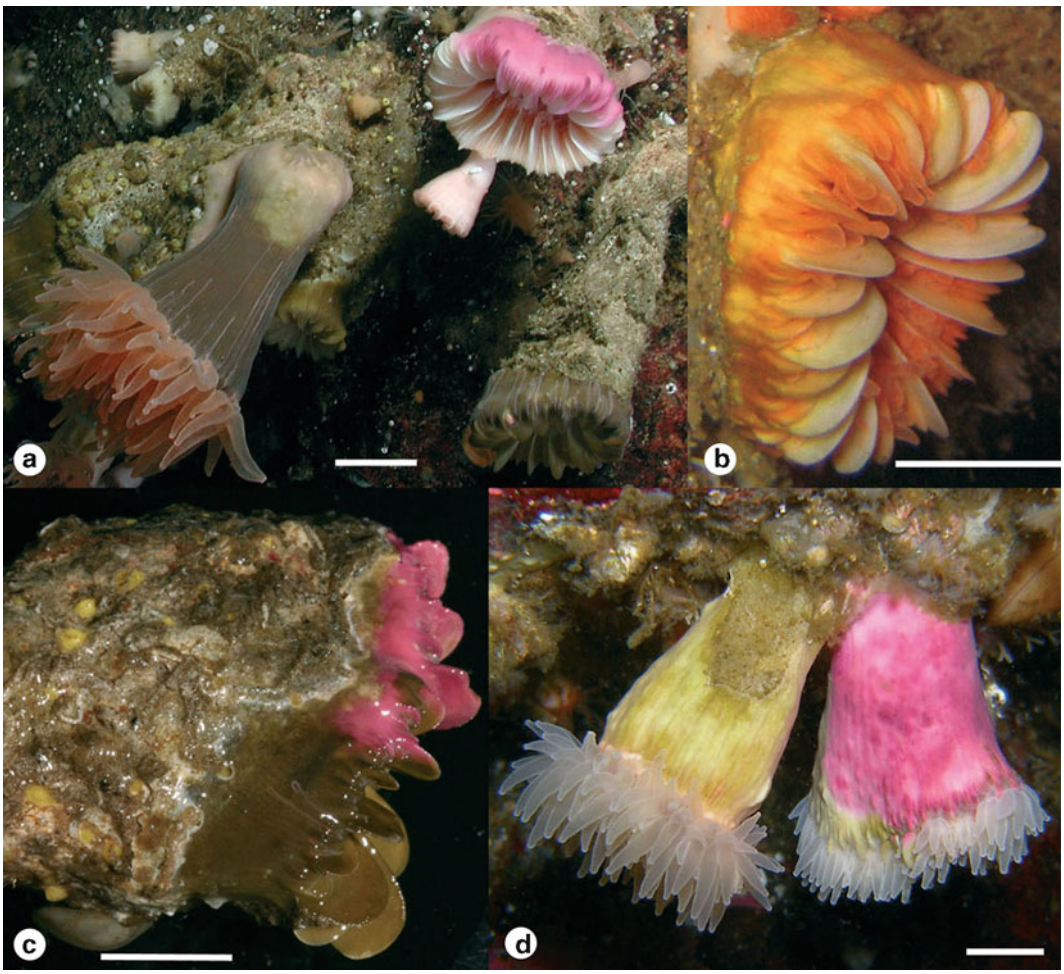

Fig. 23.16 Endolithic cyanobacterium *Plectonema terebrans* in the skeleton of the scleractinian coral *Desmophyllum dianthus* from a Chilean fjord. This cold water coral lacks endosymbiotic zooxanthellae but carries endolithic cyanobacteria and algae in its corallite skeleton. (**a**) *D. dianthus* containing the brownish, filamentous alga *Ostreobium queckettii* (the two lower specimens) or the pinkish filamentous cyanobacterium *Plectonema terebrans* (upper specimen). (**b**) *D. dianthus* appearing yellowish-orange due to the presence of a low density of *O. queckettii*. (**c**) Corallite of *D. dianthus* with pink *P. terebrans* in the upper half and the brownish *O. queckettii* in the lower half. (**d**) *D. dianthus* stained greenish by a medium density of *O. queckettii* (left) and stained pink by *P. terebrans* (right). Bars 10 mm. (Reproduced with permission from Försterra and Häussermann 2008)

Fig. 23.17 Cross-section of the ascidian *Trididemnum paracyclops* showing the green, Chl *a*- and *b*-containing endosymbiotic cyanobacterium *Prochloron didemni*, within internal cavities, and the episymbiotic *Acaryochloris marina*-like cyanobacterium (arrow) on the underside of the animal. The *Acaryochloris* contains Chl *d* which absorbs maximally in the near-infrared region, which is what remains after sunlight has passed through the animal. Bar 5 mm. (Reproduced with permission from Larkum and Kühl 2005)

two *Acaryochloris*-like symbionts in the tunic of *Lissoclinum fragile* (Lopez-Legentil et al. 2011). Epiphytic *Acaryochloris* spp. are also found on the marine red macroalga *Ahnfeltiopsis flabelliformis* (Murakami et al. 2004) and on green and brown marine macroalgae (Ohkubo et al. 2006).

Mutualistic associations between aquatic invertebrates and cyanobacteria or unicellular algae are varied and widespread, particularly in marine subtidal zones of the tropics, such as coral reefs, but are much less common in temperate marine and freshwater environments (Hirose et al. 2009b). These are often low-nutrient waters in which the photoautotrophic symbionts provide the host with a competitive advantage and the associations may make significant contributions

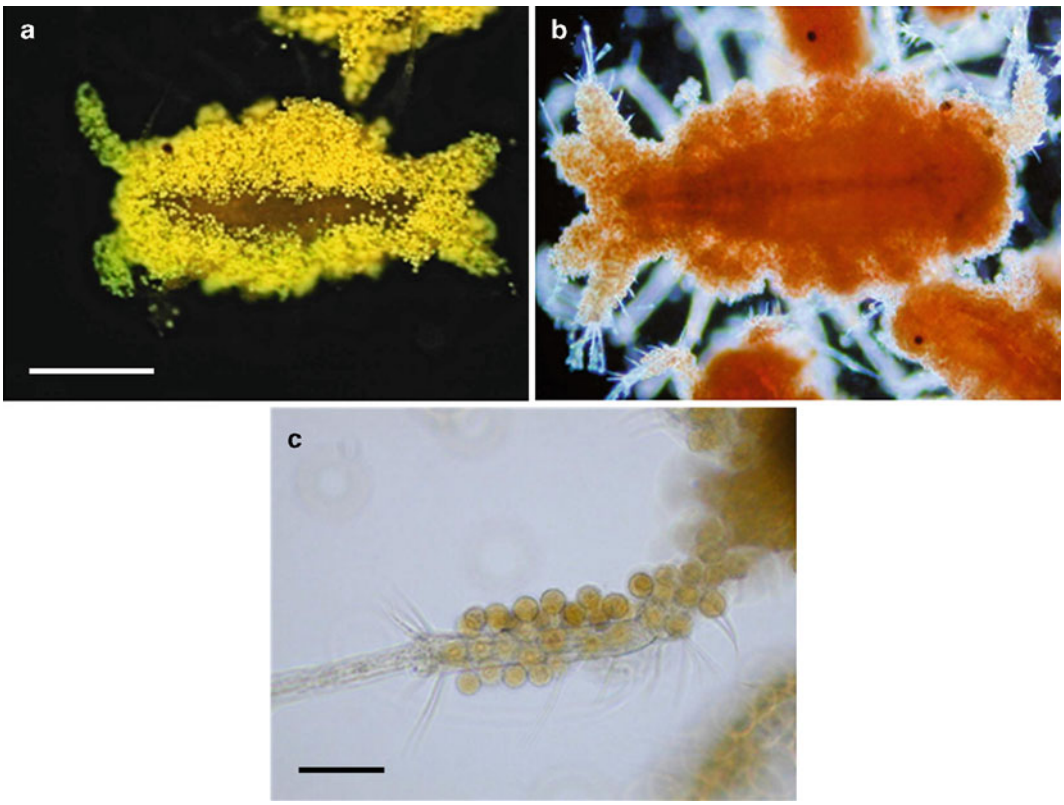

Fig. 23.18 The isopod crustacean *Santia* with episymbiotic unicellular cyanobacteria. (**a**) Epifluorescence photograph of *Santia* with episymbiotic cyanobacteria, individual cells of which can be seen on an isopod antenna in the bright field micrograph in (**c**). (**b**) Dark field image showing the reddish pigmentation of the cyanobacteria. Bars 1 mm in (**a**) and 25 μm in (**c**). (Reproduced with permission from Lindquist et al. 2005)

to the local carbon and N economy (Yellowlees et al. 2008; Venn et al. 2008). Little is known about the specific contribution of cyanobacteria-ascidian symbioses in these environments, although they may be significant. Like many marine invertebrate-bacteria associations, ascidians hosting *Prochloron* are known to produce a range of bioactive compounds, mostly cytotoxic modified peptides such as the patellamides and lissoclinamides, and the source of these is the cyanobiont (Schmidt et al. 2005; Donia et al. 2006; Piel 2006a, b; Jones et al. 2009; Donia et al. 2011a, b; Lane and Moore 2011). These bioactive compounds may act as a chemical defence, which is needed because of the sessile lifestyle of the ascidians.

23.2.5.4 Echiuroid Worms, Isopods, Hydroids and Midge Larvae

Echiuroid worms (spoon worms) are common in the intertidal zone of oceans throughout the world, where they burrow in sand or mud. Two of these soft-bodied, unsegmented worms, *Ikedosoma gogoshimense* and *Bonellia fuliginosa*, have been reported to carry cyanobacteria in their subepidermal connective tissue (Carpenter and Foster 2002; Carpenter 2002). Episymbiotic cyanobacteria are found on another animal host, marine isopods of the genus *Santia* (Fig. 23.18), found around Papua New Guinea (Lindquist et al. 2005). These are non-swimming, slow-moving crustaceans up to 5 mm in length, commonly found in groups of thousands of individuals at depths of 4–45 m. The surface of the isopods is covered by a dense carpet of 20–30 μm diameter unicellular cyanobacteria (Fig. 23.18), together with small (less than 2 μm diameter) morphologically diverse cells. There are red and brown isopods, the former being unpalatable to reef fish and the latter being palatable. The pigmentation is derived from the cyanobacteria (Fig. 23.18b), which, when examined by transmission electron microscopy, show morphological differences between palatable and unpalatable hosts (Lindquist et al. 2005). The cyanobacteria are thought to produce secondary metabolites that render the red form unpalatable and enable it to inhabit exposed, sunlit surfaces to provide the sunlight for their symbionts, which are subsequently consumed by the host. Filamentous cyanobacteria, including *Oscillatoria lutea* and *Spirulina subsalsa*, are found as epibionts on another animal host, the marine hydroid *Eudendrium racemosum* which is widely distributed in the Mediterranean Sea (Romagnoli et al. 2007). The cyanobacteria peak in abundance during the summer months.

An unusual and apparently mutualistic association is found between the cyanobacterium *Nostoc parmeloides* and larvae of the chironomid midge *Cricotopus nostocicola* (see: Adams 2000). The cyanobacterium grows as colonies attached to rocks in mountain streams of North America; the midge larvae live inside the colonies and eat the *Nostoc*, thereby gaining both a food source and protection from predation. Silk from the larvae may help to attach the *Nostoc* colonies to the rocks, but the cyanobacterium appears to benefit in other ways because the rate of photosynthesis is greater in larvae-occupied colonies than in unoccupied ones. This may be a result of the occupied colonies being able to grow further into the water flow, resulting in improved gas exchange, which in turn improves the availability of growth-limiting CO_2 and facilitates the removal of photosynthetically-generated O_2 which might otherwise inhibit N_2 fixation. The larvae appear to stimulate expansion of the *Nostoc* colonies by triggering the formation of motile hormogonia (Sect. 23.4.1.2) which can then migrate and colonise any unoccupied rock surfaces.

23.3 The Symbionts

23.3.1 Cyanobacteria

The range of cyanobacterial symbionts encompasses all morphological forms, from unicellular to filamentous non-heterocystous and filamentous heterocystous, although certain forms are favoured by particular hosts (Rasmussen and Nilsson 2002; Rasmussen and Johansson 2002). For example, most plants favour heterocystous cyanobionts of the genus *Nostoc* because members of this genus can produce the specialised, motile filaments known as hormogonia which are the infective agents in these symbioses (Meeks et al. 2002; Bergman et al. 2008a, b; Sect. 23.4.1.2). However, the ability to form hormogonia *per se* is not sufficient for symbiotic competence as hormogonia-forming cyanobacteria of genera such as *Fischerella* have never been found in plant symbioses, although they are found in lichens.

23.3.1.1 Mosses, Hornworts and Liverworts

In the epiphytic moss associations the feather moss *Pleorozium schreberi* can be colonised by *Nostoc* (DeLuca et al. 2002, 2007; Houle et al. 2006), *Stigonema* (Houle et al. 2006; DeLuca et al. 2007) and *Calothrix* (Gentili et al. 2005; DeLuca et al. 2007). When grown free-living, away from the moss, the *Nostoc* and *Calothrix* have different temperature optima for N_2 fixation, which may help explain seasonal heterogeneity for N_2 fixation in the cyanobacteria-*P. schreberi* association (Gentili et al. 2005). A N_2 fixing association can be reconstituted using both cyanobacterial isolates and non-N_2 fixing moss (Gentili et al. 2005). Two additional mosses, *Hylocomnium splendens* and *Ptillium crista-castrensis*, associate with *Nostoc* and *Stigonema* (Houle et al. 2006), and there have also been reports of cyanobacteria from other genera, including *Phormidium*, *Microcystis* and *Oscillatoria*, as epiphytes on mosses in Antarctica (Solheim et al. 2004).

The major cyanobionts of the liverworts and hornworts are *Nostoc* (Rasmussen and Nilsson 2002; Adams 2002a, b; Adams et al. 2006; Bergman et al. 2007a, b; Adams and Duggan 2008), although other hormogonia-forming cyanobacterial genera, such as *Calothrix* and *Chlorogloeopsis*, can be induced to infect liverworts in the laboratory and a single strain of *Calothrix* has been isolated from a field sample of the hornwort *Phaeoceros* (see: Adams 2002a, b). A variety of molecular techniques, including comparison of $tRNA^{Leu}$ (UAA) intron sequences (Costa et al. 2001), PCR amplification of cyanobiont DNA flanking the 16S–23S rRNA internal transcribed spacer regions, and pyrolysis mass spectrometry of isolated cyanobionts (Adams 2002a, b; Adams and Duggan 2008), have demonstrated that a wide variety of *Nostoc* strains can infect a single thallus in the field. Costa et al. (2001) found the same *Nostoc* strain shared by thalli growing 2,000 m apart, whereas others have failed to find the same strain in thalli at different sites (Adams 2002a, b; Adams and Duggan 2008). Nested PCR of the $tRNA^{Leu}$ (UAA) intron was used by Rikkinen and Virtanen (2008) to demonstrate that the primary symbiont of both *Blasia pusilla* (from Finland) and *Cavicularua densa* (from Japan) were closely related *Nostoc* strains which belonged to a specific group of symbiotic *Nostoc* strains. They concluded that, although more than one *Nostoc* genotype is often found within a single liverwort thallus, some symbiotic strains are dominant and widespread.

23.3.1.2 Cycads

Cycad cyanobionts are usually *Nostoc* although *Calothrix* have been reported on several occasions (Costa and Lindblad 2002; Rasmussen and Nilsson 2002; Gehringer et al. 2010; Thajuddin et al. 2010). Using the $tRNA^{Leu}$ (UAA) intron sequence as a genetic marker (Costa et al. 2002), Costa et al. (2004) found a single cyanobacterial strain was often present in a single coralloid root, or even in a single plant. However, a study using PCR fingerprinting with primers derived from short tandemly repeated repetitive (STRR) sequences reported multiple strains in single plants or even in single roots (Zheng et al. 2002). These varying results may reflect differences in the molecular methods used to characterise cyanobionts and it may be that the use of the $tRNA^{Leu}$ (UAA) intron sequence is more reliable than PCR fingerprinting (Costa et al. 2004). An analysis of 16S rRNA sequences and STRR PCR fingerprints of cyanobionts from three *Cycas* species from four botanical gardens in India found a diversity of *Nostoc* spp. (Thajuddin et al. 2010). A similar analysis of the 16S rRNA gene sequences of

cyanobacteria isolated from *Macrozamia* spp. throughout Australia found that the predominant *Nostoc* sp. was present in 18 root samples from 14 different *Macrozamia* spp. from a broad range of environments (Gehringer et al. 2010). The authors concluded that there was negligible host specialisation by cyanobionts in *Macrozamia* in the field.

23.3.1.3 *Gunnera*

Gunnera exerts a high level of selectivity over its symbiotic partners, only *Nostoc* spp. being found as cyanobionts, and only those strains capable of high levels of differentiation into hormogonia (Sect. 23.4.1.2; Bergman et al. 2007a, 2008a). However, this characteristic alone is not enough to guarantee a strain's symbiotic competence, as some strains forming high levels of motile hormogonia are still incapable of generating stable symbioses with *Gunnera* (Nilsson et al. 2006). While selection for *Nostoc* is highly specific, there is a growing body of work showing that *Nostoc* strains found in symbiotic relationships with *Gunnera* show high levels of genetic variability. Different *Nostoc* strains are found between different *Gunnera* plants in a similar area, and different strains of *Nostoc* are found in different species of *Gunnera* (Nilsson et al. 2000; Guevara et al. 2002; Rasmussen and Svenning 2001). Furthermore, when phylogenetic analysis is applied, it is clear that *Gunnera* cyanobionts are not all members of the same species (Svenning et al. 2005).

Closely-related *Nostoc* strains show differing abilities to infect *Gunnera* (Papaefthimiou et al. 2008a), implying that chemical signalling between the plant and bacterial cells is an essential component of the initiation of a successful symbiosis. Symbiotically-competent strains of *Nostoc* are also attracted to the crushed extracts of a variety of plants, including some which do not form symbioses (Nilsson et al. 2006). However, only symbiotically-competent species are found deep in the channels of the *Gunnera* gland structure, suggesting there is a highly specific selective mechanism within the gland itself.

23.3.1.4 *Azolla*

The *Azolla* cyanobiont was first described by Strasburger as *Nostoc* "strings" in 1873 and later re-named *Anabaena azolla*e Strasburger in 1884 (see: Pabby et al. 2004b). Although widely acknowledged as belonging to the Nostocales, there has been much debate over the correct generic assignment of the cyanobiont (Bergman et al. 2007a, b). This confusion has arisen in part because the leaf cavities are occupied by major (primary) cyanobionts (apparently unable to grow outside the symbiosis), along with minor (secondary) culturable cyanobionts (Papaefthimiou et al. 2008a, b; Sood et al. 2008a, b). The culturable cyanobionts are phenotypically and molecularly similar to each other but significantly different from the major cyanobiont.

Azolla is unique among plant-cyanobacterial associations in that the cyanobacterium never leaves its host, negating the need for re-infection of each new generation. Attempts to induce free-living strains of *Anabaena azollae* to re-infect *Azolla* cured of its cyanobiont have been largely unsuccessful and it seems impossible to cultivate the major cyanobiont separately (Lechno-Yossef and Nierzwicki-Bauer 2002; Pabby et al. 2004a), implying that one or more of the specific qualities required for free-living growth has been lost during co-evolution with the host (Rasmussen and Nilsson 2002; Sood et al. 2008b; Ran et al. 2010). It is not surprising then, that there appears to be little or no genetic diversity of the cyanobiont within a particular species of *Azolla*, indicating high host specificity, with each *Azolla* species harbouring a specific cyanobacterial strain irrespective of geographical origin.

Although the major *Azolla* symbiont has traditionally been assigned to the genus *Anabaena*, this has often been questioned, some suggesting that the cyanobiont is neither *Anabaena* nor *Nostoc* (Baker et al. 2003). Nevertheless, analyses of 16S rRNA gene sequences amplified by PCR from cyanobionts freshly recovered from *Azolla* suggest that these cyanobionts are most phylogenetically related to strains of the genus *Anabaena* (Svenning et al. 2005; Papaefthimiou et al. 2008a, b). However, in the draft genome sequence of the *Azolla* cyanobiont which has recently become available (http://genome.jgi-psf.org/anaaz/anaaz.home.html; Ran et al. 2010) the cyanobiont is referred to as *Nostoc azollae* 0708, although the closest phylogenetic relatives appear to be *Raphidiopsis brookii* D9 and *Cylindrospermopsis raciborskii* CS-505, the two filamentous cyanobacteria with the smallest known genomes (Ran et al. 2010). By contrast, the *Azolla* cyanobiont shares the highest number of protein groups with *Nostoc* PCC 7120, *Anabaena variabilis* ATCC 29413 and *Nostoc punctiforme* PCC 73102, with the last of these having the highest number of protein groups shared exclusively with *Nostoc azollae* (Ran et al. 2010). The primary cyanobiont has a small (5.49 Mb) genome, comprising one chromosome and two plasmids, and contains 5,357 coding sequences of which 3,668 have intact open reading frames while the rest are pseudogenes (Ran et al. 2010). High numbers of pseudogenes are a trait associated with endosymbionts in sheltered environments where the likelihood of encountering foreign DNA is low. The large proportion of pseudogenes (31.2%) found within all genomic functions present in the genome of the cyanobiont suggests a high level of gene erosion (Ran et al. 2010).

The taxonomic status of the minor cyanobiont population amongst *Azolla* species has received relatively little attention. The most recent analysis suggests the cyanobiont belongs to a distinct group, genetically distinct from either free-living *Nostoc* or *Anabaena* (Sood et al. 2008a) and supports previous genetic, morphological and biochemical observations

that the cyanobacteria (even the minor cyanobionts) associating with *Azolla* are peculiar to the fern (Pabby et al. 2003; Sood et al. 2008a). The continued presence of the minor symbiont in *Azolla* implies an important role in the symbiosis, but the nature of this remains a mystery.

23.3.1.5 Lichens

The genus *Nostoc* also provides many of the cyanobionts of lichens, although many other genera of both filamentous and unicellular cyanobacteria are also represented (see Rikkinen 2002 for a comprehensive list). However, until the application of molecular techniques the identification of lichen cyanobionts had relied on morphological characteristics which, even for the heterocystous cyanobacteria, are not always reliable (see for example Lücking et al. 2009), especially as morphologies can differ significantly in the free-living and symbiotic states (Sect. 23.6.2). Many cyanobionts are members of heterocystous genera including *Nostoc*, *Scytonema*, *Calothrix*, *Dichothrix*, *Stigonema*, *Tolypothrix*, and *Fischerella* (Figs. 23.9 and 23.10; Rikkinen 2002; Schultz and Büdel 2002; Sassaki et al. 2005; Schultz 2007; Muggia et al. 2011; Fedrowitz et al. 2011) and all of these can fix N_2. Unicellular cyanobionts include members of the genera *Gloeocapsa*, *Gloeothece*, *Hyella, Chroococcidiopsis, Myxosarcina* and *Synechocystis* (Fig. 23.10c), although their exact taxonomic status is often uncertain (Rikkinen 2002; Schultz and Büdel 2002; Adams et al. 2006). Some unicellular cyanobionts have been shown to fix N_2 when lichenised, although this occurs during the light, rather than in the dark as with free-living unicellular cyanobacteria (Crittenden et al. 2007).

Although some lichens have evolved means, such as soredia and isidia, for the co-dispersal of fungus and photobiont, most reproduce via sexually-produced ascospores which are not associated with photobiont cells. The germinating ascospores therefore have to locate a suitable photobiont in the environment. This need to find a suitable algal or cyanobacterial partner in the near vicinity of the germinating spore may be expected to limit the scope for specialisation of the partners since this would reduce the chances of finding the right partner (O'Brien et al. 2005; Hill 2009). Molecular phylogenetic techniques have made it possible to compare cyanobacterial strains from a wide range of lichens and look for specificity between fungus and cyanobiont. The use of the nucleotide sequences of the $tRNA^{Leu}$ (UAA) intron and 16S rDNA to compare the *Nostoc* cyanobionts of different lichens has revealed wide genetic variation (Paulsrud et al. 2000, 2001; Paulsrud 2001; Rikkinen 2002; Oksanen et al. 2002). These earlier molecular phylogenetic studies found that cyanobionts could be shared between related hosts such as members of the Nephromataceae (Paulsrud et al. 2000; Summerfield et al. 2002; Rikkinen 2002, 2003; Rikkinen et al. 2002; Lohtander et al. 2003; Wirtz et al. 2003). Later studies showed that symbiont sharing was common even between unrelated hosts and not just in lichens from extreme environments (such as the Antarctic) where the choice of photobiont might be low (Wirtz et al. 2003), but also in those from temperate regions (O'Brien et al. 2005; Summerfield and Eaton-Rye 2006).

The two primary factors which might influence the selection of the cyanobiont by the lichen fungus are habitat ecology and fungal taxonomy, yet distinguishing between these using molecular phylogenetic techniques has proved difficult and controversial. Earlier studies concluded that it was primarily the taxonomic identity of the lichen, rather than its geographic origin, that determined its choice of cyanobiont (Paulsrud et al. 2000; Summerfield et al. 2002). However, subsequent studies concluded that geographic origin was the more important factor. For example, unrelated cyanolichens were shown to share the same cyanobiont and these lichens often formed characteristic communities or "guilds" that shared a common habitat, so epiphytic cyanolichens associated with old-growth forests formed the *Nephroma* guild and many predominantly terrestrial cyanolichens formed the *Peltigera* guild (Rikkinen 2002, 2003; Rikkinen et al. 2002). The cyanobionts within these guilds are closely related and form two clades, referred to by Rikkinen et al. (2002) as *Nostoc* groups A and B, which are cyanobionts of, respectively, the *Nephroma* and the *Peltigera* guilds.

This concept of "pools" of symbiotically-competent cyanobacteria being available for lichenisation by mycobionts in the vicinity gained further support from the work of Summerfield and Eaton-Rye (2006), who concluded that there was no correlation between mycobiont diversity and cyanobiont choice in the *Pseudocyphellaria* species examined. Following more comprehensive surveys using sequences of $tRNA^{Leu}$, 16S rDNA, *rbcL* and *rbcX* as genetic markers, Stenroos et al. (2006) and Myllys et al. (2007) concluded that, although many lichen fungi were indeed selective towards their *Nostoc* cyanobionts, this correlated primarily with the fungal taxa, rather than the terrestrial or epiphytic nature of the lichens. However, a different conclusion was reached by Elvebakk et al. (2008) who examined phylogenetic patterns of the *Nostoc* symbionts of both bi- and tri-partite lichens of *Pannaria* from seven countries in northern and southern hemispheres. They broadly supported the idea that selectivity of *Nostoc* strains resulted primarily (although not exclusively) from ecological influences, but there was a transition between the *Nephroma* and *Peltigera* guilds (Elvebakk et al. 2008). Further support for lichen guilds was presented by Lücking et al. (2009) based on their phylogenetic analysis of what were thought to be *Scytonema* cyanobionts in a range of tropical lichens. The cyanobionts, which were shared by at least four genera of lichen mycobionts, formed a distinct clade which was more closely related

to *Nostoc*, *Anabaena*, *Fischerella* and *Hapalosiphon* than to *Scytonema*, and which they named *Rhizonema*.

In summary, although there is still some dispute about whether it is ecology or genetics which most influences the mycobiont's choice of cyanobacterial partner, it is clear that mycobionts are selective in their choice of cyanobionts, whereas many cyanobionts associate with a range of often unrelated mycobionts.

23.3.1.6 *Geosiphon pyriformis*

In *Geosiphon pyriformis* only *Nostoc* strains, usually referred to as *Nostoc punctiforme*, can be cyanobionts (Kluge et al. 2002; Adams et al. 2006). In its natural environment *Geosiphon* is found together with the liverwort *Blasia*, the hornwort *Anthoceros*, and the moss *Dicranella* (Kluge et al. 2002). Both *Blasia* and *Anthoceros* have their own *Nostoc punctiforme* symbionts and these can be recognised and incorporated by *Geosiphon* (Adams et al. 2006). There are also endosymbiotic bacteria (referred to as bacteria-like organisms, BLOs) within the fungus and, unlike the cyanobiont, these are not surrounded by a host membrane (Adams et al. 2006).

23.3.1.7 Diatoms

Comparison of *nifH*, *hetR* and 16S rRNA sequences of the *Richelia intracellularis* cyanobionts of the diatoms *Hemiaulus hauckii* from the North Atlantic and *Rhizosolenia clevii* from the North Pacific has shown that they are different species (Foster and Zehr 2006). This work also confirmed that the cyanobiont of *Chaetoceros*, previously referred to as both *Richelia intracellularis* and *Calothrix rhizosoleniae*, is closely related to, but distinct from, the *Richelia* symbionts of *Hemiaulus hauckii* and *Rhizosolenia clevii*. Even within the Mediterranean Sea two phylogenetically-discrete populations of *R. intracellularis* have been found in *Hemiaulus hauckii*, seemingly kept apart by local water circulation patterns (Bar Zeev et al. 2008).

Phylogenetic analysis of the 16S rDNA sequence of the coccoid cyanobiont of the diatom *Climacodium frauenfeldianum* from the Atlantic and Pacific Oceans has shown that it is closely related to the N_2 fixing *Cyanothece* ATCC 51142 (Carpenter and Janson 2000; Janson 2002) and to free-living unicellular N_2 fixing cyanobacteria found in the tropical North Atlantic Ocean (Falcón et al. 2002). Similarly, genome sequence analysis of the cyanobiont (the so-called spheroid bodies) of the marine pennate diatom *Rhopalodia gibba* has confirmed that it also has a close relationship to *Cyanothece* ATCC 51142 (Prechtl et al. 2004; Kneip et al. 2008). However, the genome of this cyanobiont has undergone significant changes, including the elimination, fusion and truncation of genes, and the accumulation of deleterious mutations in genes for cell wall biosynthesis, confirming the speculation that it is an obligate endosymbiont and that it must be transmitted vertically (Kneip et al. 2008).

23.3.1.8 Dinoflagellates, Radiolarians and Tintinnids

A range of unicellular cyanobacterial symbionts has been reported in members of the dinoflagellate genera *Ornithocercus*, *Histioneis*, *Parahistioneis*, *Citharistes*, *Dinophysis* and *Amphisolenia* (Fig. 23.4; Carpenter 2002; Foster et al. 2006a, b; Tarangkoon et al. 2010). RT-PCR analysis of 16S rRNA sequences of cyanobionts from *Citharistes*, *Ornithocercus* and *Histioneis* has shown them to be related to *Prochlorococcus*, although additional 16S rRNA sequences from *Citharistes* spp. were related to *Synechococcus* (Foster et al. 2006b). The cyanobionts of the two radiolarians, *Spongostaurus* and *Dictyocoryne truncatum*, are thought to be related to *Prochlorococcus* (Foster et al. 2006a, b). The open-ocean tintinnid *Codonella* hosts unicellular cyanobacterial symbionts closely related to *Synechococcus* (Carpenter and Foster 2002; Foster et al. 2006b). It seems that many diverse open-ocean hosts, encompassing dinoflagellates, tintinnids and radiolarians, house cyanobionts related to the unicellular, free-living marine *Synechococcus* and *Prochlorococcus*, indicating a relatively low degree of specificity in these symbioses (Foster et al. 2006b).

23.3.1.9 Sponges

Cyanosponges contain both unicellular and filamentous cyanobacteria as symbionts (Hentschel et al. 2006; Usher 2008). Unicellular cyanobacteria include *Aphanocapsa feldmannii*, *A. raspaigellae*, *Synechococcus* spp., *Prochloron* spp. and *Synechocystis trididemini*, whereas filamentous cyanobacteria include *Oscillatoria spongeliae*, which occurs as closely-related strains in a wide range of sponges (Thacker and Starnes 2003; Hentschel et al. 2006; Hill et al. 2006; Usher et al. 2006; Thacker et al. 2007; Zhu et al. 2008; Usher 2008; Erwin et al. 2012). 16S rRNA studies have revealed that *Aphanocapsa feldmannii* symbionts are in fact *Synechococcus* (Hentschel et al. 2002; Gómez et al. 2004; Usher et al. 2004a). Analysis of high molecular weight DNA from the sponge *Halichondria* has identified sequences showing high homology with cyanobacteria from the unicellular genus *Synechococcus* and the filamentous, heterocystous genus *Nostoc* (Ouyang et al. 2010).

However, the most prevalent sponge cyanobiont is probably *Synechococcus spongiarum* which has been isolated from taxonomically-diverse hosts from wide-ranging geographical locations and appears to be specifically adapted to the host sponges (Usher et al. 2004b; Steindler et al. 2005; Thacker 2005; Oren et al. 2005; Erwin and Thacker 2007, 2008a, b; Hardoim et al. 2009). Comparison of cyanobiont 16S rDNA sequences has provided invaluable information on cyanobiont identity and diversity. In general, specific sponges

host specific cyanobionts, both across wide geographical locations and when sponges hosting alternative cyanobionts are close by (Hentschel et al. 2002; Usher 2008). For example, different sponges have been shown to always host the same distinct strain of *Oscillatoria spongeliae* (Thacker and Starnes 2003; Ridley et al. 2005a) and the cyanobionts of *Chondrilla australiensis* from tropical and temperate regions are from the same clade (Usher et al. 2004a). Individual sponges occasionally host two or more cyanobionts (Usher et al. 2004a; Ridley et al. 2005b; Steindler et al. 2005).

Relatively few sponges have been studied with respect to the mode of transmission of cyanobionts, which could occur either vertically, directly from parent to offspring, or horizontally, by the offspring obtaining the cyanobiont from the environment (Taylor et al. 2007; Usher 2008). Vertical transmission has been shown in the oviparous sponge *Chondrilla australiensis* (Usher et al. 2001, 2005; Usher 2008) and the viviparous *Diacarnus erythraeanus* (Oren et al. 2005). Occasional horizontal transfer may also occur, possibly facilitated by the expulsion of cyanobionts along with eggs during spawning (Taylor et al. 2007; Usher 2008). Remarkably, cyanobacteria have been found in both eggs (Usher et al. 2004b) and sperm (Usher et al. 2005) of *Chondrilla australiensis*. However, even in this case occasional females do not vertically transmit their cyanobionts at all and offspring may acquire new cyanobionts from the water (Usher et al. 2005). Symbiotic cyanobacteria have also been seen in the oocytes of the marine sponge *Chondrilla nucula* (Maldonado 2007) and in buds produced by the marine sponge *Tethya orphei* (Gaino et al. 2006).

23.3.1.10 Corals

The coccoid cyanobionts of the coral *Montastreae cavernosa* are most closely related to either *Synechococcus* or *Prochlorococcus* in the order Chroococcales (Lesser et al. 2004, 2007). Among the partial *nifH* sequences obtained by PCR amplification of DNA from tissues of *Montipora capita* and *M. flabellata* were some most closely resembling those of the cyanobacterial genus *Myxosarcina* (Olson et al. 2009). In addition, unidentified cyanobacterial DNA sequences have been detected in the metagenome of the bacterial community from the coral *Porites compressa* (Thurber et al. 2009). Terminal restriction fragment length polymorphism analysis of the microbial population of the coral *Montastraea annularis* identified an abundant cyanobacterium (cyanobacterium CD1C11) normally associated with coral black band disease (BBD), but in this case found in many samples of healthy coral (Klaus et al. 2007). BBD affects corals worldwide and is characterised by a microbial mat dominated by cyanobacteria that were initially identified as *Phormidium corallyticum*. However, later analysis of BBD mats from three different locations revealed them to contain cyanobacteria from at least three different taxa of the order Oscillatoriales (Frias-Lopez et al. 2003; Myers and Richardson 2009). It isn't clear if cyanobacterium CD1C11 is epibiotic or endobiotic on healthy *Montastraea annularis*.

23.3.1.11 Ascidians

The most frequent cyanobionts in the colonial ascidians of the family Didemnidae are unicellular *Prochloron* spp., which contain chlorophyll *a* and *b* but lack phycobilin pigments (Griffiths 2006; Hirose and Hirose 2007; Hirose et al. 2009b; Lopez-Legentil et al. 2011). However, the unicellular, coccoid cyanobacterium *Synechocystis trididemni*, shown by molecular phylogenetic analysis to be a close relative of *Prochloron* (Shimada et al. 2003), has been reported in *Trididemnum* species (Münchhoff et al. 2007; Lopez-Legentil et al. 2011). *Synechocystis trididemni*, or a closely-related species, is also the dominant cyanobiont in *Trididemnum clinides* from Japan, but this ascidian harbours two additional cyanobionts, a possibly new non-*Prochloron* species and a filamentous, non-heterocystous cyanobacterium, possibly an *Oscillatoria* sp. (Hirose et al. 2009b).

Phylogenetic analysis of *Prochloron* spp. from a wide range of didemnid ascidians in widespread geographical locations has revealed little genetic variation and no relationship between phylogeny and geographic location, implying a low level of host specificity (Münchhoff et al. 2007). The *Prochloron*-didemnid symbiosis is generally considered to be obligate for both partners because *Prochloron* has never been grown in culture (apart from one unconfirmed early report) and has only very rarely been seen free-floating away from the host. Indeed, in many photosymbiotic didemnids the cyanobiont is passed vertically from mother to larva, although the exact mechanism of transmission varies in different didemnids (Hirose 2000; Hirose et al. 2005, 2006c; Hirose and Hirose 2007; Kojima and Hirose 2010). However, *Prochloron* 16S rRNA gene sequences have been found in living stromatolites in an environment free of ascidians, implying that *Prochloron* can exist free of its host (Burns et al. 2004). Similarly, recent sequencing of the genome of the *Prochloron didemni* symbiont from *Lissoclinum patella* revealed a complete set of metabolic genes, implying that the cyanobacterium can reproduce outside the host (Donia et al. 2011).

Colonial ascidians from the Mediterranean Sea have recently been found with a complex epibiotic population of cyanobacteria from genera *Planktothricoides, Synechococcus, Phormidium* and *Myxosarcina* (Martinez-Garcia et al. 2011). Cyanobacteria resembling the chlorophyll *d*-containing *Acaryochloris marina* are found as epibionts on the undersides of the didemnid ascidians *Lissoclinum patella*, *Trididemnum paracyclops* and *Diplosoma similis*, but are not found within the ascidian tissue (Kühl et al. 2005; Larkum and Kühl 2005). However, two *Acaryochloris marina*-like cyanobacteria have recently been found in the tunic of both adults and larvae of *Lissoclinum fragile* from the Bahamas (Lopez-Legentil et al. 2011).

23.3.1.12 Isopods

The surface of small, marine crustacean isopods of the genus *Santia* is covered by a dense carpet of 20–30 μm diameter unicellular cyanobacteria, together with small (less than 2 μm diameter) morphologically-diverse cells (Fig. 23.18; Lindquist et al. 2005). Comparison of PCR-amplified DNA-dependent RNA polymerase complex (rpoC1) clones from symbionts has revealed strong similarity with *Synechococcus*, *Synechocystis* and *Prochlorothrix* (Lindquist et al. 2005). The authors concluded that the *Synechococcus* were the smaller cells found alongside the large symbiont and were environmentally derived, whereas the large-celled symbionts were *Synechocystis* and were probably vertically transmitted.

23.3.2 Bacteria

Apart from *Azolla*, which is described below, little is known of the possible involvement of bacteria in cyanobacterial symbioses. In *Gunnera*, microorganisms other than cyanobacteria are excluded from the symbiotic structures occupied by the cyanobionts, whereas in sponges for example, complex populations of bacteria are also present.

The leaf cavities in all *Azolla* species house a substantial range of endosymbiotic bacteria, often referred to as bactobionts or eubionts. Bacteria are also found at the shoot apex, in the indusium chambers of both mega and microsporocarps and germlings, and even inside *Azolla* root and stem tissues (Bergman et al. 2007a; Zheng et al. 2009a, b). Older leaves in general harbour larger bacterial communities, although the number and type of bacteria present varies depending on the *Azolla* species. In some cases the bacterial population has been found to be equal to the number of cyanobacterial cells present. Estimates suggest that only 1% of the bacteria are cultivable, implying that the isolates identified to date represent only part of the bacterial population able to enter the *Azolla*-cyanobacterium associations (Zheng et al. 2009a, b). The significance of these bacteria, which have also been found in cyanobiont-free *Azolla* spp. (reviewed by Zheng et al. 2009a), is unclear, although their presence throughout the life cycle of *Azolla* would imply an important contribution to the association. There is evidence that some may fix N_2 and also contribute to the polysaccharide-rich mucilaginous matrix associated with *Azolla* leaf cavities and possibly also to that found in the indusium chambers of the sporocarps and in the sporeling (Zheng et al. 2009a, b). Early work has also shown that cultured *Arthrobacter* isolated from *Azolla* secrete auxin when supplied with the precursor tryptophan, raising the possibility that the bacterial endosymbionts release the plant hormone in the symbiosis with *Azolla* (Lechno-Yossef and Nierzwicki-Bauer 2002).

23.4 Host-Cyanobacteria Interactions Prior to Infection

23.4.1 What Makes a Successful Cyanobiont?

In the plant symbioses successful cyanobionts possess two significant characteristics – they are capable of forming motile hormogonia, which are essential for successful plant invasion, and heterocysts, which are required for N_2 fixation and the establishment of a functional symbiosis. Hormogonia are particularly important in the plant associations because the immotility of the host means that the cyanobacteria must find the plant. By contrast, motile hosts such as diatoms and sessile filter-feeders such as sponges can locate and capture potential symbionts from the surrounding water. Indeed, for many of the non-plant cyanobacterial symbioses, hormogonia are not required and the cyanobionts are often incapable of forming hormogonia or heterocysts; their role is then usually the provision of fixed carbon for the association.

For hormogonia to successfully locate and invade the plant structures that will house the symbiotic colonies they must possess at least two characteristics in addition to motility. Firstly, they must be able to adhere to, and perhaps specifically recognise, the host plant surface; external filamentous protein structures known as pili are essential for this. Secondly, they must be able to sense chemoattractants released by the host. Finally, in addition to the ability to form heterocysts and hormogonia plant cyanobionts need to be facultative heterotrophs. In many of the plant symbioses the cyanobiont is found in locations (e.g. cycad roots and *Gunnera* stem glands) that receive little, if any, light and so it must be able to grow heterotrophically on fixed carbon supplied by the plant partner.

23.4.1.1 Heterocysts

Many cyanobionts, especially those of plants, are heterocystous cyanobacteria from the genus *Nostoc* and their primary role is to provide fixed nitrogen for the symbiotic partnership, although in some cases, such as two-membered cyanolichens, they also provide fixed carbon. Heterocysts are the sites of N_2 fixation, providing a suitable microoxic environment for nitrogenase to function. They develop in response to N limitation and their frequency in symbiosis is often greatly elevated above that in free-living cyanobacteria (Sect. 23.6.2.2) resulting in an increased rate of N_2 fixation. However, heterocysts are important for another reason – they are produced by all cyanobacteria of the families Nostocaceae and Stigonemataceae, and it is these cyanobacteria that are capable of the production of a particular type of motile hormogonia, the formation of which is characterised by rapid cell division resulting in a decrease in cell size (Sect. 23.4.1.2). It is these hormogona that are the infective agents in many plant symbioses.

23.4.1.2 Hormogonia

Cyanobionts from genera such as *Nostoc*, which are sessile for most of their life cycles, have the ability to convert immotile vegetative filaments into short, specialised motile filaments known as hormogonia, that serve as both a means of dispersal and as the infective agents in most cyanobacteria-plant symbioses (Meeks 2003, 2009; Meeks and Elhai 2002; Bergman et al. 2007a). There are a variety of environmental factors that trigger hormogonia development (Meeks et al. 2002; Meeks and Elhai 2002; Meeks 2009) and these include chemicals released by plants (Sect. 23.4.2). Once inside the plant the hormogonia lose motility and form the heterocysts that are essential for the establishment of a successful, N_2 fixing symbiosis.

In heterocystous cyanobacteria the first visible event in hormogonia development is a round of very rapid cell divisions, which occurs in all cells of a filament, without cell growth, resulting in a large decrease in cell volume (Meeks and Elhai 2002; Bergman et al. 2008a; Meeks 2009). Subsequently, the vegetative cell-heterocyst junctions become narrowed and break, resulting in the loss of the heterocysts and the release of the short interheterocyst sections of filament, which at the same time acquire motility and are now hormogonia. Because hormogonia lack heterocysts they are only a transient stage in the *Nostoc* life-cycle, soon loosing motility and returning to vegetative growth. At this point they begin to develop heterocysts, initially from the terminal cell at each end of the hormogonium (at which point the hormogonium is sometimes referred to as a primordium), but later at intercalary positions once vegetative cell division increases the length of the filament. The surface of *Nostoc punctiforme* hormogonia is covered with pili (fimbriae) which are clearly important for the infection process and may be involved in motility, host recognition and surface attachment (Duggan et al. 2007).

23.4.1.3 Pili

The cell surface of hormogonia is covered with filamentous, protein structures known as Type IV pili (Tfp) which are found in many bacteria and are involved in a wide range of processes including adhesion, pathogenesis, DNA uptake and motility (Mattick 2002; Nudleman and Kaiser 2004; Burrows 2005). In *N. punctiforme* the non-motile vegetative filaments lack pili, whereas the surface of motile hormogonia is covered with abundant, peritrichously-arranged pili (Duggan et al. 2007). Tfp are involved in the motility of some unicellular cyanobacteria (Bhaya 2004), but it isn't known if they are also involved in the gliding motility of filamentous cyanobacteria, or of hormogonia. Mutation of two *N. punctiforme* genes, homologues of *pilT* and *pilD* which are thought to be involved in Tfp structure and function, has adverse effects on the ability of the mutant hormogonia to infect the liverwort *Blasia* (Duggan et al. 2007). Because of inconsistent motility in the wild-type hormogonia it isn't possible to determine from the data of Duggan et al. (2007) if the reduced hormogonia symbiotic competence in the Tfp mutants is due to loss of motility (and hence chemotaxis; next section), or to loss of some other trait, such as host recognition or adhesion to the plant surface. Further support for the involvement of pili in symbiotic interactions comes from a proteomic and transcriptional analysis of *Nostoc* hormogonia formation which revealed strong upregulation of a homologue of *pilQ* (Klint et al. 2006), upstream of which in the *Nostoc punctiforme* genome are three ORFs homologous to *pilM*, *pilN* and *pilO*, all of which are involved in pilus formation (Meeks et al. 2001).

23.4.1.4 Chemotaxis

The production of motile hormogonia is essential for the efficient infection of many plant hosts, yet some *Nostoc* strains produce motile hormogonia but do not show symbiotic competency, so there must be additional characteristics required to ensure successful infection. One such characteristic is likely to be the ability to sense and respond to plant-derived chemoattractants to enable the hormogonia to locate and invade host symbiotic structures. This sensory capacity is reflected in the gene expression changes that accompany the development of hormogonia, induced by either N starvation or exposure to hormogonia-inducing factors (Sect. 23.4.2) released by plants (Campbell et al. 2007, 2008). Within 24 h of inducing hormogonia formation in *N. punctiforme* the transcription of 944 genes is upregulated and 856 downregulated; this is fivefold greater than the number of transcriptionally-active genes in N_2 fixing cultures or in those developing akinetes (Campbell et al. 2007). A majority of the up-regulated genes encode proteins for signal transduction and transcriptional regulation, with additional ones including genes encoding putative chemotaxis proteins and genes involved in pilus biogenesis (Meeks et al. 2001; Klint et al. 2006; Campbell et al. 2007). The genome of *N. punctiforme* has 3–5 copies each of the genes encoding homologues of the chemotaxis-related proteins CheA, CheB, CheW, CheD and CheR (Meeks et al. 2001). This chemotactic ability is probably particularly important in plants such as *Gunnera* and the cycads in which the symbiotic tissue is in dark locations and the hormogonia must override their natural positive phototaxis.

Chemotaxis is clearly important in the *Blasia*-cyanobacteria symbiosis because the N starved liverwort releases a very effective chemoattractant (Adams and Duggan 2008), although this is unlikely to be specific to *Blasia* as non-host plants such as *Trifolium repens* (Nilsson et al. 2006) and germinating wheat seeds (Adams and Duggan 2008) can also release hormogonia chemoattractants. No chemical identification of any of these chemoattractants has been made, although it has been suggested that they may be sugar-

based molecules; indeed, simple sugars such as arabinose, glucose and galactose do attract hormogonia, with arabinose being the most effective (Nilsson et al. 2006). Arabinose is found at high levels in *Gunnera* stem gland mucilage, possibly released from arabinogalactan proteins or arabinan-containing pectins by the extracellular enzyme ARAf, the gene for which is expressed at higher levels in stem tissue containing glands compared with that lacking glands (Khamar et al. 2010). The presence of high levels of arabinose in the mucilage ensures that hormogonia are attracted into the gland channels, and from there into the deeper tissues. Inside the mature gland tissue high levels of the reducing sugars glucose, fructose and sucrose suppress further hormogonia formation (Khamar et al. 2010).

It might be thought that hormogonia and chemotaxis are not needed in the *Azolla* symbiosis because the fern does not require *de novo* infection, yet filaments resembling hormogonia are found at the apical regions of the plant (Zheng et al. 2009a; Sect. 23.5.4). This hormogonia-like phase is without doubt important in the *Azolla* symbiosis as it has been retained during a long evolutionary history of association with the plant. These filaments are motile and possibly guided to the symbiotic cavity and the megasporocarps (Sect. 23.5.4) by chemotaxis (Zheng et al. 2009b). In support of this, preliminary annotation of the draft genome sequence of the obligate cyanobiont in the *A. filiculoides* symbiosis (http://genome.jgi-psf.org/anaaz/anaaz.home.html) reveals a substantial number of genes with potential involvement in signal perception and transduction of that signal into a developmental or behavioural response, as well as genes that may encode a pilus-related motility apparatus.

23.4.1.5 Other Characteristics

Apart from motility and chemotaxis there must be additional, more subtle characteristics of hormogonia that influence plant infection. Mutations in *cyaC*, which encodes adenylate cyclase, the enzyme responsible for the biosynthesis of the intracellular messenger adenosine 3′, 5′-cyclic monophosphate (cAMP), alter the efficiency of infection of *Blasia* by *Nostoc punctiforme*, implying that cAMP may play a role in infection (Adams and Duggan 2008; Chapman et al. 2008). However, the situation is complex because mutations in two different domains of the multi-domain CyaC adenylate cyclase result in different infection phenotypes in *Blasia*, with one mutant infecting at 25% of the wild-type frequency, but the other at 300–400% of the wild-type, even though both possess similar cellular cAMP levels at 25% of the wild-type (Chapman et al. 2008). There are no differences between the two mutants in terms of the frequency of hormogonia produced in the presence of *Blasia*, or the motility or piliation of the hormogonia. These data imply that cAMP *per se* is not involved in symbiotic competency, and that the contrasting infection phenotypes of the mutants are a result of unknown behavioural differences of the mutant hormogonia in response to plant signals.

Further evidence that the behaviour of hormogonia, as much as their abundance, influences host infection comes from a mutant of *Nostoc punctiforme* inactivated in the gene *sigH*, which encodes an alternative sigma subunit of RNA polymerase (Meeks 2003). Transcription of this gene is induced by a hormogonia-inducing factor (HIF; Sect. 23.4.2) from *Anthoceros*, yet a mutant inactivated in *sigH* produces the same frequency of HIF-induced hormogonia as the wild-type, although they are up to fivefold more infective of the hornwort than wild-type hormogonia (Meeks and Elhai 2002; Meeks 2003).

23.4.2 Signalling Between Potential Partners

It is of clear benefit to a plant host to stimulate hormogonia production in potential cyanobionts and so improve the chances of infection. To this end plants, particularly when starved of combined nitrogen, release factors that trigger the formation of hormogonia (hormogonia-inducing factor, HIF; see below) and act as chemoattractants. To improve their chances of infection host plants also produce a factor that prolongs the motile hormogonial phase. For example, a component of *Gunnera* stem gland mucilage increases the hormogonial stage from 20 to 40 h to several weeks (see: Bergman et al. 2007a). However, once inside a symbiotic cavity the plant releases a hormogonia-repressing factor (HRF) which is dominant over HIF and ensures that vegetative growth, heterocyst production and N_2 fixation are resumed (Sect. 23.6.1). Hormogonia-inducing factors have been found in the hornwort *Anthoceros punctatus* (Meeks et al. 2002; Meeks and Elhai 2002; Meeks 2003, 2009), cycads and the angiosperm *Gunnera* (Rai et al. 2000; Bergman et al. 2008a) and the liverwort *Blasia* (Watts 2000; Adams 2002a, b; Adams and Duggan 2008).

The chemical identity of HIF is not known, but available evidence implies that is will be different in different plants. The HIF of *Anthoceros punctatus* is a small, heat-labile compound released during N starvation (Meeks and Elhai 2002; Meeks 2003, 2009). Mutants of *Nostoc punctiforme* showing increased responsiveness to *Anthoceros* HIF also show a greater initial frequency of infection of the hornwort than the wild-type. The frequency of HIF-induced hormogonia in *N. punctiforme* is reduced by mutation of the gene *ntcA*, encoding the global transcriptional regulator NtcA (Herrero et al. 2004) and the resulting hormogonia fail to infect *Anthoceros* (Wong and Meeks 2002).

In the *Gunnera* symbiosis the structure of the gland, the presence of stipulate tissue and the presence of exuded mucus are all accommodations by the plant to encourage the presence of *Nostoc* hormogonia. However, the plant places a strong selection on the invading cyanobacteria and it is likely that a

system of positive and negative controls ensures that only cyanobacteria of the genus *Nostoc* are found within the stem glands. Evidence of the influence that *Gunnera* exerts on cyanobiont physiology is seen in *Nostoc* protein changes induced by the plant. Enhanced expression of three genes (*hieA*, *hieB* and *hieC*) in symbiotically-competent *Nostoc* is stimulated by *Gunnera* gland mucilage (Liaimer et al. 2001). The genes encode an outer membrane glycoprotein, a potential signalling compound and a protein that may be involved in adaptation of *Nostoc* to an acidic environment such as that in *Gunnera* mucilage (Liaimer et al. 2001; Bergman et al. 2007a). More recently, proteomic analysis has identified 38 proteins differentially expressed in symbiotic *Nostoc* cells compared with free-living cells (Ekman et al. 2006). Four are associated with the cell surface and are upregulated in the symbiotic organism; one of the four contains fasciclin-like repeats which have been implicated in symbiotically-important proteins in a lichen symbiosis (Paulsrud and Lindblad 2002).

Once *Nostoc* cells are within the *Gunnera* gland larger molecules may be responsible for signalling: either diffusible soluble molecules or larger, non-diffusible molecules found within the extracellular slime of the cyanobacterium or attached to the cell surfaces of either the cyanobacterium or the plant cells. Potential signalling compounds are the arabinogalactan proteins (AGPs) which are a very diverse group of proteoglycans generally found associated with the extracellular matrix of plant and some algal cells. Their structure, function and expression patterns have been well reviewed elsewhere (Seifert and Roberts 2007). AGPs have been associated with the initiation (and potentially the maintenance) of the *Alnus-Frankia* symbiosis, being found at the symbiotic interface in the root nodules (Berry et al. 2002). Early evidence has shown AGPs to be associated with *Gunnera* gland mucilage (see: Bergman 2002; Bergman et al. 2007a), but their role in the formation and maintenance of the *Gunnera-Nostoc* symbiosis has never been extensively investigated.

23.4.3 Other Important Factors

23.4.3.1 Lectins

The fungal partner of lichens and the plant host in bryophyte and *Azolla* symbioses produce lectins that can recognise and bind to sugars on the surface of symbiotic *Nostoc* strains (Lehr et al. 2000; see also: Rikkinen 2002; Adams et al. 2006). The cyanolichens *Peltigera canina* and *Leptogium corniculatum* produce an arginase that acts as a lectin by binding to a polygalactosylated urease in the *Nostoc* cell wall (Diaz et al. 2009; Vivas et al. 2010). Such lectins could be involved in fungus-partner recognition in lichens (Lehr et al. 2000; Elifio et al. 2000; Legaz et al. 2004; Sacristán et al. 2006), although proof of this is lacking; a model for the signalling pathways that might be involved has been proposed by Rikkinen (2002). In the *Geosiphon* symbiosis the only stage of the *Nostoc* life cycle that is incorporated by the fungus is the immotile primordial stage, which occurs immediately after the motile hormogonia stage (Sect. 23.5.6). Primordia are labelled by a mannose-specific lectin ConA, whereas heterocysts and hormogonia have different lectin-binding patterns (Kluge et al. 2002; Adams et al. 2006). This implies that alterations in *Nostoc* extracellular glycoconjugates could be important in recognition.

23.4.3.2 Proteins

Several proteins have been identified in the *Nostoc* proteome as being potentially important in symbiosis, but no temporal or spatial location has yet been attributed to them within a symbiotic system. One appears to be a two-component signal transduction system based on two putative proteins found in the proteome of diazotrophically-grown *Nostoc* PCC. 73102 (Ran et al. 2007). Another pair of proteins identified in the same study, a polysaccharide biosynthesis/export protein and a phosphomannomutase, were also suggested to be symbiotically important, although their exact roles remain to be elucidated. Another study identified a protein with 84% amino acid homology to a cyclodextrin glucosyltransferase, and hence the gene encoding the protein was named *cgt* (Wouters et al. 2003). These authors proposed several possible roles for Cgt in the fixation of N_2 under heterotrophic conditions and in the synthesis of the gelatinous material that coats filaments of *Nostoc* vegetative cells.

23.5 Host Structures and Their Infection

With the exception of the fungal associations (lichens and *Geosiphon pyriformis*) and to some extent *Gunnera*, cyanobacteria occupy existing structures in the host. Although cyanobacteria are photoautotrophs, many are also facultative photo- or chemoheterotrophs, which enables them to occupy locations that receive little or no light.

23.5.1 Hornworts and Liverworts

Bryophyte symbiotic structures are present in uninfected plants and so are not unique to the symbiotic state. In the liverwort *Blasia* the cyanobacteria occupy hemispherical structures known as auricles, on the underside of the thallus (Fig. 23.2c, d), whereas in hornworts such as *Anthoceros* and *Phaeoceros* they are found within the thallus in slime (mucilage) cavities (Fig. 23.2a) connected to the ventral surface of the thallus via mucilage clefts (Meeks 2003; Adams et al. 2006; Bergman et al. 2008a). Although mucilage clefts superficially resemble stomata, they are not thought to be related (Villarreal and Renzaglia 2006). The clefts form by

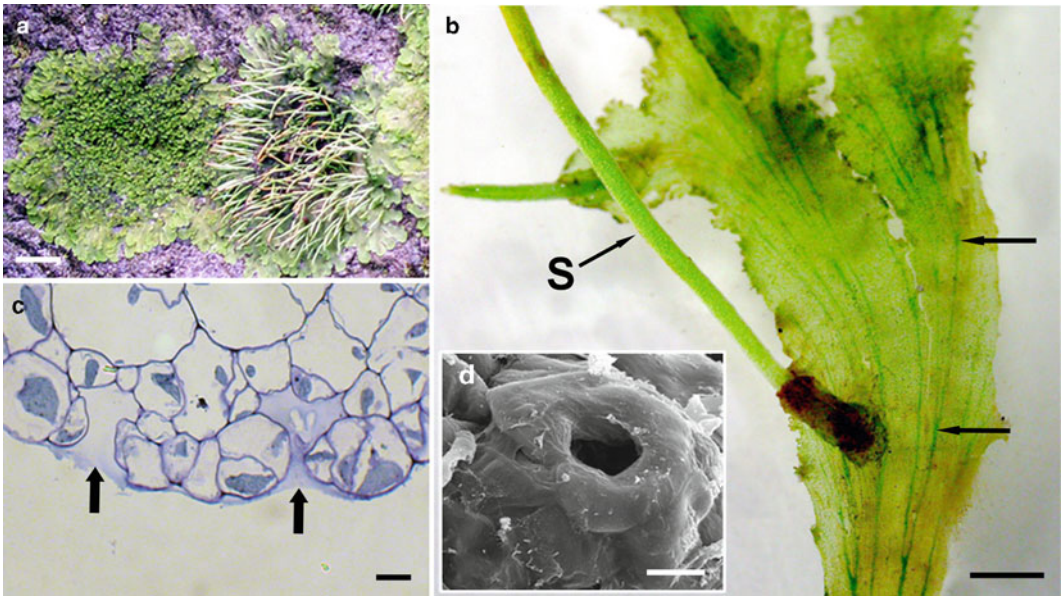

Fig. 23.19 The hornwort *Leiosporoceros dussii* with symbiotic *Nostoc*. (**a**) To the left is a young rosette and to the right an older thallus with numerous upright sporophytes. (**b**) Dark green *Nostoc* 'strands' (arrows) can be seen within the thallus, parallel to the main axis. *S* sporophyte. (**c**) Light micrograph of a nearly transverse section of two mucilage clefts (arrows), which provide the entry point for cyanobacterial infection; the *Nostoc* filaments subsequently spread through channels created by the separation of cells along their middle lamellae. (**d**) Scanning electron micrograph of a mucilage cleft. Bars 10 mm in (**a**), 2 mm in (**b**), 15 μm in (**c**) and 20 μm in (**d**). (Reproduced with permission from Villarreal and Renzaglia 2006)

apparently random separation of adjacent epidermal cells on the ventral side of the thallus; the slime cavity then develops beneath the cleft (Renzaglia et al. 2000). The *Blasia* auricle develops from a three-celled mucilage hair which undergoes extensive elaboration to form the dome-shaped auricle, which then becomes infected by *Nostoc* (Renzaglia et al. 2000). In the hornwort *Leiosporoceros dussii* (Fig. 23.19a) the cyanobacteria occupy mucilage-filled 'canals' (Fig. 23.19b) formed by the separation of plant cell walls along their middle lamellae (Villarreal and Renzaglia 2006). These canals run parallel to the main axis of the thallus and as they elongate they bifurcate to form an integrated network, enabling the *Nostoc* to spread internally throughout the thallus (Villarreal and Renzaglia 2006). By contrast, the isolated nature of the discrete colonies in *Blasia*, *Anthoceros* and *Phaeoceros*, means that each individual symbiotic structure must become infected from the outside.

It is motile hormogonia that enter hornwort slime cavities and the auricles of *Blasia*, but once inside the plant the priority is to establish a N_2-fixing colony, so motility is lost and heterocyst development is initiated (Adams 2002a, b; Adams et al. 2006). Entry to the hornwort slime cavities is through the mucilage clefts (Figs 23.2a and 23.19c, d) and there are interesting parallels between this and the likely infection route in a possible symbiotic relationship between the primitive, extinct land plant *Aglaophyton major* and an *Archaeothrix*-type filamentous cyanobacterium (Taylor and Krings 2005). From fossil evidence, cyanobacteria are thought to have entered the plant via stomatal pores, colonising the substomatal chambers and from there spreading throughout the outer cortical tissue, where they can be seen in fossil specimens. This hypothetical infection process is similar to that in the living hornwort *Leiosporoceros dussii* described above (Villarreal and Renzaglia 2006).

Although the establishment of a functional, N_2 fixing symbiosis requires the development of heterocysts, mutants incapable of heterocyst development are still able to infect *Anthoceros punctatus* (Wong and Meeks 2002). For example, strains inactivated in *hetR* (Wong and Meeks 2002), the primary driver of heterocyst differentiation (Golden and Yoon 2003; Zhang et al. 2006), and *hetF* (Wong and Meeks 2002), which is involved in the regulation of *hetR* transcription and the localisation of HetR to developing heterocysts (Wong and Meeks 2001), infect the hornwort as efficiently as the wild-type although they are incapable of supporting growth of the plant because they lack heterocysts (Wong and Meeks 2002). By contrast, inactivation of *ntcA*, which encodes the global nitrogen regulator NtcA (Flores and Herrero 2005), completely prevents infection even though the mutant produces motile hormogonia (Wong and Meeks 2002).

23.5.2 Cycads

It is the coralloid roots of cycads that house their cyanobionts (Costa and Lindblad 2002; Lindblad and Costa 2002;

Bergman et al. 2007a, 2008a). These roots constitute approximately 1–3.6% of plant biomass and display negative geotropism, growing outwards and upwards from the tap root, sometimes breaking the surface of the soil. The cyanobionts are found in a mucilage-filled zone between the inner and outer cortical layers (Fig. 23.3). The formation of coralloid roots occurs in plants in the absence of cyanobacteria and so is not a response to infection. However, entry of the cyanobacteria into the coralloids does trigger significant morphological change, including an increase in root diameter and elongation of the cells linking the inner and outer cortical layers, possibly to facilitate nutrient exchange (Costa and Lindblad 2002). How the cyanobacteria gain entry to the coralloid root is unclear, but possible points of entry include lenticels, or breaks in the dermal layer (Costa and Lindblad 2002; Bergman et al. 2007a) and it has been suggested that bacteria and fungi in the cycad rhizosphere may cause local degradation of the cell wall, enabling the cyanobacteria to penetrate the root (Lobakova et al. 2003).

23.5.3 *Gunnera*

In *Gunnera* the site of entry for potential cyanobionts is glands found at the base of each leaf stem (petiole) and covered in red tissue and a sticky mucilage (Fig. 23.5). The mucilage is secreted in large amounts by glands in mature plants and plays vital roles in the symbiosis, including the induction and chemoattraction of hormogonia (Bergman 2002; Bergman et al. 2007a). In larger species of *Gunnera* these glands are surrounded by 'stipulate' tissue, made up of long leaf-like fronds (Fig. 23.4), and it has been suggested that this tissue type is required to allow *Nostoc* access to the plant glands, which may be several metres above the soil (Benson and Margulis 2002). The gland itself is made up of 6–9 outward-facing papillae, one forming a central stem with the others surrounding it (Fig. 23.5b). These glands are seen in all *Gunnera* plants, including those grown under sterile conditions and not brought into contact with any cyanobacteria. Formation of the glands appears to be triggered by low environmental N and hence their formation is related to the plant's requirement for fixed nitrogen (Chiu et al. 2005). Furthermore, the structural features of the glands appear vital to the formation of the symbiosis because removal of the outlying papillae, leaving only the central structure, prevents the plant from forming a stable symbiosis. However, only one of the outer papillae is needed to re-establish the capacity for symbiosis (Uheda and Silvester 2001). This suggests that the cyanobacteria travel down the channels between the papillae (Fig. 23.6a, b) to the bottom of the glands, and the papillial structures have an essential role in allowing the cyanobacteria to invade the cells at the base of the gland (Fig. 23.6c, d).

Once the hormogonia have reached the interior of the *Gunnera* stem gland they invade the plant cells by an undetermined mechanism (Bergman 2002; Bergman et al. 2007a). Localised mitotic activity near the infection site might be caused by the phytohormone indole-3-acetic acid (IAA) which symbiotic cyanobacteria are capable of producing (Sergeeva et al. 2002). It is thought that these dividing cells are the ones that become infected (Bergman et al. 2007a). Once cell invasion has taken place, the cell wall appears normal in host cells containing the cyanobionts. At this stage the gland channels disappear, preventing any further infection. Delineated clusters of cells containing the cyanobiont appear, and are bordered by layers of uninfected plant cells. The formation of such "organs" is unusual, and may suggest further extracellular signalling between the plant and the cyanobacterium. Within the *Gunnera* cells, the cyanobacteria form clusters and fill the space, although they never enter the cell cytoplasm as they never penetrate the plant cell plasmalemma, which is thought to act as the interface between the plant and its cyanobiont, where all nutrients (travelling both ways) are exchanged (Bergman 2002; Bergman et al. 2007a).

23.5.4 *Azolla*

Azolla has overlapping leaves consisting of two lobes each approximately 1 mm in length (Fig. 23.7a, b), the thick, aerial dorsal lobe containing chlorophyll and the partially-submerged, thinner achlorophyllous ventral lobe serving as a float. Dorsal lobes have an extracellular ovoid cavity, approximately 0.3 mm in length, formed by an infolding of the adaxial epidermis during development (van Hove and Lejeune 2002a; Lechno-Yossef and Nierzwicki-Bauer 2002; Bergman et al. 2007a, b; Zheng et al. 2009a). The cavity, which is at first open to the outside, becomes closed as the leaf matures, and is occupied by a highly complex prokaryotic community, including 2,000–5,000 cyanobiont cells and numerous heterotrophic bacteria (bactobionts or eubionts), that remains intimately associated with the plant throughout its life cycle. In mature leaf cavities the bactobionts and cyanobacteria are found at the periphery, immobilised within a polysaccharide-rich mucilaginous material between internal and external envelopes, which are probably of plant origin and function in metabolite exchange (van Hove and Lejeune 2002a, b; Lechno-Yossef and Nierzwicki-Bauer 2002). The central region of the cavity is free of symbionts and is gas-filled (Lechno-Yossef and Nierzwicki-Bauer 2002). A pore in the adaxial epidermis of the leaf cavity connects it with the external environment and probably functions in gas exchange. The pore is lined with teat-shaped cells which may function in water repulsion and serve as a physical barrier, blocking the entry of particles and organisms and preventing the cyanobacteria bactobionts from leaving (Veys et al. 2000, 2002; see also Zheng et al. 2009a).

As many as 25 simple hairs, together with two primary branched hairs, protrude into the mucilage layer around the periphery of the leaf cavity and function in metabolite/signal exchange between the host and its symbionts (Lechno-Yossef and Nierzwicki-Bauer 2002; Pereira and Carrapiço 2007; Zheng et al. 2009a). Indeed, both the cytoplasm and chloroplasts of branched hair cells contain higher glutamine synthetase levels than those in cyanobiont-free plants (Uheda et al. 2004), implying that these cells are involved in the assimilation of N released by the cyanobiont. Branched hairs are associated with the cavity throughout its development and facilitate the inoculation of developing leaf cavities with both cyanobacteria and bactobionts. By contrast, simple hairs increase in number as a cavity matures. The hairs are also present in cyanobiont-free cavities and are fundamental to the maintenance of the *Azolla*-cyanobacteria association; their role, along with other structural features of this association, has been reviewed recently (Zheng et al. 2009a).

The permanent association of *Azolla* and its cyanobiont is possible because the fern's reproduction processes are inextricably linked with transfer of cyanobacteria to each new plant generation. *Azolla* is able to reproduce both sexually (a complex process involving sporocarp production) and asexually (the main form of reproduction). Asexual reproduction is rapid, with a doubling time of 2 days or less under optimal laboratory conditions and involves fragmentation of branches from the main plant stem (rhizome). The apical regions of *Azolla* contain rapidly-dividing undifferentiated cyanobacterial filaments resembling hormogonia, which are introduced into the leaf primordium before development of the leaf and new leaf cavity are complete (Zheng et al. 2009b). Primary branched hairs form bridge-like structures at the apical meristems, promoting the partitioning of the cyanobacterial inoculum into the young leaf cavities (Zheng et al. 2009a, b). Sexual reproduction in *Azolla* is less common and appears to be triggered by adverse environmental conditions, plant density and light intensity (reviewed by Lechno-Yossef and Nierzwicki-Bauer 2002; Pabby et al. 2004a). *Azolla* sporophytes produce two morphologically-distinct sporocarps, the male microsporocarps and the female megasporocarps. The latter contain a single megasporangium (consisting of a single megaspore and the megaspore apparatus) and a colony of *Anabaena*.

The process by which *Anabaena* is packaged into the developing megasporocarp pairs, thereby securing its horizontal transfer to the next plant generation, has attracted considerable interest. Zheng and co-workers (2009b) have examined the development of entrapped cyanobacteria in the developing megasporocarps of *A. microphylla* fronds located at the second, fourth and fifth branch points (approximately 5, 10 and 17 days old, respectively). A population of small-celled motile cyanobacterial filaments resembling hormogonia enter the developing sporocarps through a pore at the top of the indusium chamber (Fig. 23.7e), presumably guided by chemotaxis towards the developing megasporocarp pairs found in the apical region of the sporophyte (Zheng et al. 2009b). Following entry of the hormogonia into the developing megasporocarp, the cells of the filaments undergo synchronous conversion to spore-like resting cells known as akinetes (Fig. 23.8; Zheng et al. 2009b), with the result that the cyanobacterial population in the mature megasporocarp consists primarily of large, individual akinetes (Figs. 23.7e and 23.8f). One of the triggers for akinete development in free-living cyanobacteria is phosphorus limitation and this may be the trigger in the megasporocarp, as polyphosphate granules are rarely observed in the cyanobacteria during their transfer to the megasporocarp (Zheng et al. 2009b), possibly as a result of impaired phosphate uptake (Ran et al. 2010). The megasporocarp pore closes following the colonization phase, thereby preventing further entry of cyanobacteria and the other bacteria that are associated with the hormogonia-like filaments in the apical region (Zheng et al. 2009b). Given the status of the akinete as a survival structure it is not surprising that the mature megasporocarp, awaiting germination, harbours the cyanobiont in its own resting phase. This represents an example of the synchronous development that occurs between the cyanobiont and its host, a strategy that has evolved to maintain this unique plant-cyanobacterial association. Similarly, fertilisation and embryogenesis are followed by akinete germination to produce metabolically-active vegetative filaments which, with the assistance of the cotyledonary hairs, are introduced into the embryonic leaf (Zheng et al. 2009a).

23.5.5 Lichens

Unlike any other cyanobacterial symbiosis the morphology of lichens (Fig. 23.9) bears no resemblance to that of the free-living partners, and although the cyanobiont can influence thallus development, it is mostly the mycobiont that determines the morphology and chemistry of the lichen (Rai and Bergman 2002; Rikkinen 2002; Sanders 2006). So, the fungal thallus that will house the photobionts exists only in the symbiotic state. Lichens generally, although not exclusively, fall into three morphological classes referred to as crustose (a thin, crust-like layer), foliose (leaf-like) and fruticose (branched). In bipartite cyanolichens the cyanobacterium generally forms a continuous layer beneath the upper cortex (Fig. 23.10d), but can be dispersed throughout the thallus (Fig. 23.10b, c). In the tripartite lichens, the green algal photobiont occurs as a layer throughout the thallus, but the cyanobacterium is isolated in specialised structures known as cephalodia, either within the thallus or on its surface (Fig. 23.10a; Rikkinen 2002; Adams et al. 2006). Cephalodia only form when cyanobacteria are present

and the formation of each is a new event (Rai et al. 2000). These structural arrangements are not always so simple; for example in chimeroid lichens, known as photosymbiodemes, green algae and cyanobacteria are the primary photobionts in different parts of the thallus, with a gradual transition between the two, often along a light or humidity gradient (Rikkinen 2002). By contrast, the 'jelly lichens' of the Collemataceae produce homoiomerous thalli, lacking a distinct photobiont layer, and owe their gelatinous habit to the copious extracellular polysaccharide produced by the *Nostoc* cyanobionts (Wedin et al. 2009).

23.5.6 *Geosiphon pyriformis*

In *Geosiphon pyriformis* the specialised fungal structures (bladders; Fig. 23.11b, c) that house the cyanobionts are only produced in response to the presence of suitable cyanobacteria. The *Nostoc* cyanobiont is intracellular, and is incorporated into the fungal hyphae by endocytosis (Kluge 2002; Kluge et al. 2002; Adams et al. 2006). The *Nostoc* must be at the "primordium" stage (Sect. 23.4.1.2) of its life cycle to be recognised by the fungus; primordia are formed when motile hormogonia lose their motility and produce the first heterocysts. The process of incorporation begins when the tips of the fungal hyphae encounter *Nostoc* primordia and the fungal wall softens and bulges outwards, surrounding usually 5–15 *Nostoc* vegetative cells (Fig. 23.11a), although existing heterocysts are cut off and are not endocytosed (Kluge et al. 2002; Adams et al. 2006).

Each infected hypha swells to form a 2 mm long, pear-shaped multinucleate bladder, that is coenocytic with the fungal mycelium and contains the cyanobacteria (Fig. 23.11b, c). Bladders without endosymbionts are never found, implying that formation of the bladder is a specific response to the endocytosis of the cyanobacteria. The bladders are highly vacuolated and at the basal end, which is beneath the soil surface, are milky white in appearance due to the presence of numerous lipid droplets (Fig. 23.11c). The apical three quarters of the bladder is above the soil surface and is dark in appearance due to the presence of the *Nostoc* symbionts (Adams et al. 2006). The symbionts occupy a cup-shaped compartment known as the symbiosome, formed by invagination of the fungal plasma membrane (Fig. 23.11c). The symbiotic interface is the space between the host membrane and the *Nostoc* cell wall and this is filled with chitin, resembling the symbiotic interface between the fungal wall and the plant plasma membrane in arbuscular mycorrhizas (Adams et al. 2006). Heterocysts are present in the *Nostoc* within the bladders, although their frequency is not elevated as it is in many cyanobacterium-plant symbioses (Sect. 23.6.2.2) and this reflects the primary role of the cyanobiont in *Geosiphon*, which is to provide fixed carbon for the fungus (Kluge 2002).

23.5.7 Diatoms and Dinoflagellates

In the symbiosis between the diatom *Rhizosolenia* and the heterocystous cyanobacterium *Richelia intracellularis* the cyanobiont is found extracellularly in the host's periplasmic space, between the plasmalemma and the frustule (Carpenter 2002; Bergman et al. 2007a). By contrast, in *Rhopalodia* the unicellular cyanobiont is located in the cytoplasm but separated by a host membrane (Rai et al. 2000; Janson 2002; Bergman et al. 2007a).

In the dinoflagellate-cyanobacteria symbioses the cyanobiont location varies in the different genera of dinoflagellates (Fig. 23.14; Carpenter 2002); in *Ornithocercus*, *Citharistes*, *Histioneis* and *Parahistioneis* the cyanobacteria are located externally to the host cytoplasm, whereas in *Amphisolenia* they are within the cytoplasm. In *Ornithocercus* the cyanobionts are located between the upper and lower cingular list, whereas in *Histioneis* they are in a chamber on the girdle floor (Jyothibabu et al. 2006).

23.5.8 Sponges

Sponges vary in size from less than 1 cm to several metres and are sessile filter feeders which collect food from the large volumes of water that pass through an aquiferous canal system. The sponge body is hollow, the wall consisting of a layer of pinacocyte cells on the outside and a layer of choanocytes on the inside, separated by the gelatinous mesohyl. It is the beating of flagella on the choanocytes that circulates water through the sponge. The supporting skeleton, consisting of a fibrous protein and spicules made of silica or calcium carbonate, is found in the mesohyl. In larger sponges the wall becomes pleated and the mesohyl is thickened and embedded with many interconnected choanocyte chambers. The inner part of the sponge is known as the endosome (or choanosome) and this is protected from strong currents and high light intensities by the outer layers. Food particles are taken up by phagocytosis in the choanocyte chambers located in the endosome.

Symbiotic cyanobacteria are usually intercellular, but sometimes occur in specialised vacuoles called cyanocytes (Usher 2008). In general, cyanobacteria are found in the outermost few millimetres of the sponge, where light availability is greatest, whereas the internal mesohyl contains a complex mixture of symbiotic heterotrophic and autotrophic bacteria (Hentschel et al. 2006). However, there are exceptions such as *Oscillatoria spongeliae* found abundantly in the mesohyl, but not in the surface layers of the sponge *Lendenfeldia chondrodes* (Ridley et al. 2005b), and *Synechococcus* present in the endosome of *Tethya aurantium* (Thiel et al. 2007). In the latter example it seems

that radiating silica spicules act as a fibre-optic system to conduct light into the deeper sponge tissue (Brümmer et al. 2008).

23.5.9 Ascidians

In the cyanobacteria-ascidian symbioses the cyanobiont can be found on the colony surface, in the peribranchial and common cloacal cavity (Hirose and Hirose 2007; Hirose et al. 2009a, b; Kojima and Hirose 2010), or in the tunic (Hirose et al. 2006b; Hirose and Nakabayashi 2008), depending on the host species. In the case of *Trididemnum clinides* the three cyanobionts differ in their distribution in the host tunic, type-A (possibly a novel non-*Prochloron* species) being found predominantly near the colony surface, together with small numbers of type-C (a possible *Oscillatoria*), whereas type-B (possibly *Synechocystis didemni*) is found throughout the tunic (Hirose et al. 2009b). This segregated distribution is thought to result from the cyanobionts occupying the location most suited to their different pigment contents.

Cyanobionts can be either intracellular or extracellular depending on the didemnid and on the cyanobiont location within the animal. For example, in *Lissoclinum punctatum* the *Prochloron* cells found in the tunic are mostly intracellular, whereas those in the peribranchial and common cloacal activities are extracellular (Kojima and Hirose 2010). *Prochloron* cells attach to the tunic wall because of the adhesive nature of the wall lining (Hirose and Fukuda 2006; Hirose and Nakabayashi 2008). The tunic of photosymbiotic didemnids is usually transparent, although they do contain UV-absorbing substances (such as mycosporine-like amino acids, MAAs), calcareous spicules and pigmented tunic cells (Hirose et al. 2004; Hirabayashi et al. 2006) which may create an ideal light environment for the cyanobionts (Hirose et al. 2009b). For example, *Didemnum molle* colonies in shallow water (10 m) have high MAA concentration and low spicule density to block UV without attenuating photosynthetically-active radiation, whereas colonies in deeper water (20 m) have much lower concentrations of MAAs (Hirose et al. 2006a).

23.6 Host-Cyanobiont Interactions Post-infection

Cyanobacteria in symbiosis, particularly with plants, frequently show morphological and physiological modifications including repression of hormogonia development, increased cell size, reduced growth rate, increased heterocyst frequency and N_2 fixation, and depressed N assimilation and carbon dioxide fixation.

23.6.1 Hormogonia Repression and Cell Division Control

23.6.1.1 Hormogonia Repression

Although it is to a host plant's advantage to induce hormogonia formation in potential symbionts, once the cyanobacterium has infected the plant it is essential that hormogonia formation is repressed, to facilitate heterocyst development and N_2 fixation. To this end a water-soluble hormogonium repressing factor (HRF), which is dominant over the hormogonia-inducing HIF signal, is released into the symbiotic cavity in the hornwort *Anthoceros punctatus* (and presumably also in liverworts and *Gunnera*) to inhibit hormogonia formation even in the presence of HIF (Meeks and Elhai 2002; Meeks et al. 2002; Meeks 2003). In *Nostoc punctiforme* HRF induces expression of *hrmA*, which has no significant sequence homology with genes in the databases but which is part of a *hrmRIUA* operon that is similar in sequence to sugar uronate metabolism operons of other bacteria (Campbell et al. 2003; Meeks 2003). The expression of *hrmA* is also induced by the plant flavonoid naringin (Cohen and Yamasaki 2000). A strain mutated in *hrmA* forms hormogonia in the presence of HRF. Hormogonia repression is achieved through the sugar-binding transcriptional repressor HrmR, which prevents any new rounds of development (Campbell et al. 2003). There is evidence that fructose (possibly converted to a signalling metabolite) may be involved in the repression of hormogonia formation (Ungerer et al. 2008). Indeed, hormogonia formation is repressed by sucrose, glucose and fructose, the latter two of which are present at high concentrations in *Gunnera manicata* mature stem gland tissue, repressing further hormogonia formation and thereby enabling heterocyst differentiation and N_2 fixation (Khamar et al. 2010).

The *hrm* operon may be involved in symbiotic systems other than *Anthoceros* because aqueous extracts from fronds of *Azolla pinnata* and *A. filiculoides* are potent inducers of *hrmA* expression in *N. punctiforme* strain UCD 328 (Cohen et al. 2002). Moreover deoxyanthocyanin, the coloured pigment in *Azolla* leaves that increases during the winter months and under phosphate limitation, producing a distinct reddish hue, acts in synergy when mixed with other plant-derived compounds, resulting in a significant increase in *hrmA* induction (Cohen et al. 2002). Mature *Azolla* frond tissue is redder than actively-growing apical regions which may imply a role for deoxyanthocyanins as an HRF component.

23.6.1.2 Cell Division Control

In many cyanobacterial symbioses, particularly those involving plants, the host grows much more slowly than its symbiont. Therefore, to maintain a stable symbiosis and avoid being rapidly out-grown the host must regulate the growth of the cyanobiont. Strategies include the blocking of cell division,

physical confinement (by restricting the number and size of the symbiotic structures) and restriction of the nutrient supply (Rai et al. 2000; Ekman et al. 2006; Bergman et al. 2007a). In the case of the hornwort *Anthoceros* the growth rate of symbiotically-associated *Nostoc* can be up to tenfold slower than when in the free-living state (Meeks 2003). Although the mechanism is not known, *Anthoceros* can also regulate *Nostoc* colony biomass and N_2 fixation rate to ensure that the rate of N_2 fixation per unit of plant tissue remains constant when, for example, the formation of new colonies is inhibited by penicillin, or growth is stimulated at elevated light intensity and CO_2 (Meeks and Elhai 2002; Meeks 2003).

In *Azolla*, growth of the cyanobiont can be differentially regulated in specific regions of the plant. Growth rates of both partners in the *Azolla* association are at their maximum in the apical regions and decrease linearly along the axis away from the apex, towards the mature regions of the host (Bergman et al. 2007a, b). When plant growth is inhibited with cycloheximide (a specific inhibitor of protein synthesis in eukaryotes), the rapid cell division occurring in the cyanobiont present in the plant apex also stops. The cyanobiont population (number of cells per cavity) and cell size also increase from apical to older regions of the fern (leaf numbers 1–15). Although the cell size continues to increase in much older regions of the plant (leaf numbers 15–28), the population of the cyanobiont becomes constant (see: Bergman et al. 2007a, b). This regulation of growth may not apply so stringently to the primary *Azolla* cyanobiont, which is readily detected in cyanobacterial preparations from crushed whole plant tissues using molecular probes (see: Meeks 2009). By contrast, the secondary (and culturable) cyanobiont is not detected using the same approach, leading to the suggestion that these cyanobionts are present at a level significantly lower than that of the primary cyanobiont, and their growth is under stringent control by the host. The apparent lack of growth control over the primary cyanobiont might be a reflection of some of the adaptations that have evolved for life in obligate symbiosis (Meeks 2009).

Evidence of host control over growth and cell division of cyanobionts is also found in sponges in which cyanobacterial abundance seems to be proportional to the number of sponge cells (Taylor et al. 2007). How such control is effected isn't clear, although restriction of the cyanobiont's access to essential nutrients and the sequestration of carbon fixed by the cyanobiont are possible mechanisms (Taylor et al. 2007).

23.6.2 Morphological Modifications

23.6.2.1 Cell Morphology

Although the morphology of cyanobacterial symbionts in sponges seems not to be affected by the host or its biogeographic location (Usher et al. 2006), it is more common for the cell morphology of the cyanobacteria to be altered in symbiosis. For example, filamentous growth can become aseriate, and cells are often enlarged and altered in shape. Such changes often display an increasing gradient of severity from the youngest to the oldest symbiotic tissues. Cell enlargement and shape irregularity are apparent in the vegetative cells of *Nostoc* associated with hornworts (Meeks and Elhai 2002). In cyanolichens filamentous cyanobacteria such as *Scytonema* and *Calothrix* can become unicellular and *Nostoc* cell size increases (Rai and Bergman 2002). In the *Geosiphon* symbiosis, once the *Nostoc* cells are engulfed by the fungal hyphae they seem to undergo a period of stress, when they become deformed and their photosynthetic pigments become bleached (Fig. 23.11a; Kluge et al. 2002; Adams et al. 2006). Some cells die at this stage, but within 3 days the remainder recover and enlarge to approximately six times their free-living volume. *Nostoc* ultrastructure seems little changed inside *Geosiphon*, although the outer membrane is difficult to discern in electron micrographs and the heterocyst cell wall is thinner than usual, possibly indicating a reduced concentration of oxygen within the bladders (Adams et al. 2006).

The enlarged-cell phenotype is also seen in the *Azolla* leaf cavity where the volumes of both the vegetative cells and heterocysts of the cyanobiont are approximately four times greater than those of free-living cyanobacteria (see: Zheng et al. 2009a). Other morphological differences include vegetative cell shape, filament shape and pigmentation, and heterocyst cell shape and frequency (Pabby et al. 2003, 2004b; Papaefthimiou et al. 2008a; Sood et al. 2008a, b). The minor *Azolla* cyanobionts, which are able to grow under free-living conditions, retain the ability to utilise external supplies of sugar and show the modified morphological and physiological characteristics of symbiotic growth, including larger cells, increased frequency of heterocysts, increased N_2 fixation and increased respiration, leading to the suggestion that sugar metabolism rather than plant-derived factors may regulate some of the changes associated with symbiosis (Ungerer et al. 2008).

In general, the *Nostoc* cyanobionts of cycads show little morphological or structural change from free-living strains (Costa and Lindblad 2002), although there have been reports of cyanobionts with vegetative cells and heterocysts showing considerable degradation of the peptidoglycan layer (Baulina and Lobakova 2003a, b). However, it is not clear whether such cells are functional or are in various states of senescence.

23.6.2.2 Heterocyst Frequency

A characteristic of cyanobacterial symbioses involving a photosynthetic host is a significant increase in cyanobiont heterocyst frequency above that in the free-living state (Table 23.1), with a consequent enhancement of N_2 fixing capacity. There is often an increasing gradient of heterocyst

Table 23.1 Heterocyst frequency and the characteristics of glutamine synthetase in symbiotically-associated cyanobacteria. (Table compiled from Meeks (2009) and Adams (2000) and references therein)

Host	Heterocyst frequency (%)	Glutamine synthetase		Form of combined nitrogen released (% released)
		Amount of protein (%)	Specific activity (%)	
Cycads	17–46	100	100	Glutamine/citrulline? (ND)
Gunnera	20–60	100	70	NH_4^+ (90)
Hornworts and liverworts	25–45	86–100	~15	NH_4^+ (80)
Azolla	26–45	5–40	~30	NH_4^+ (40)
Lichens:				
Bipartite	4–8	<10	<10	NH_4^+ (90)
Tripartite	10–55	<10	<10	NH_4^+ (90)

Heterocyst frequency is expressed as a percentage of the sum of heterocysts plus vegetative cells. The range of frequencies reflects the increasing heterocyst frequency found with increasing age of symbiotic tissue. Typical frequencies for free-living cyanobacteria are 4–10%. Glutamine synthetase is expressed as the amount of protein (as a percentage of that in the same cyanobacterium growing in the free-living state) or the specific activity (as a percentage of that in the same cyanobacterium growing in the free-living state). The form of N released by the cyanobacterium to the host is given, with (in parenthesis) the amount of N released by the cyanobiont as a percentage of the total N_2 fixed. ND not determined

frequency from approximately 10–15% in the most actively growing region of the plant (such as the root tip in cycads) to 60% in old symbiotic tissue, although the highest rate of N_2 fixation often occurs at an intermediate heterocyst frequency. There is also a loss of symbiont CO_2-fixing capacity because of the reduction in vegetative cell frequency and the inability of heterocysts to fix CO_2, but this is compensated by a supply of fixed carbon from the host (or the green algal symbiont in the case of tripartite lichens) and a shift to a photo- or chemoheterotrophic mode of nutrition in the cyanobiont.

In the hornworts and liverworts cyanobionts have 6–10-fold higher heterocyst frequencies than in the free-living state (Table 23.1; Meeks 2003). Although some of these additional heterocysts may be non-functional, the elevated frequency nevertheless enhances the N_2 fixing capacity of the symbiotic colonies (Meeks and Elhai 2002; Meeks et al. 2002). Within the colonies heterocysts can be difficult to recognise because they often lose some of the morphological traits that distinguish them from vegetative cells, including their regular shape and thickened cell walls (Meeks and Elhai 2002; Adams and Duggan 2008).

In the *Azolla* cyanobiont heterocysts are absent in the very young leaves at the stem apex and first appear in filaments as they become enclosed within the symbiotic cavity in leaf 1. Heterocysts increase in frequency with successive leaves, reaching a maximum of 25–45% of the cell population by leaves 12–15 (Table 23.1; see: Lechno-Yossef and Nierzwicki-Bauer 2002; Pabby et al. 2004a). Despite the high heterocyst frequencies, double, triple or higher numbers of contiguous heterocysts are as infrequent as they are in free-living cultures (Meeks and Elhai 2002). Exogenous supplies of fixed nitrogen have limited inhibitory effects on heterocyst frequency and nitrogenase activity in the *Azolla* association (Meeks and Elhai 2002; Meeks 2009), raising the possibility that a plant-derived signal stimulates heterocyst development in symbiosis (see below). The cyanobionts in the coralloid roots of cycads also show a gradient of heterocyst frequency, the lowest (16.7%) being found at the growing root tip and the highest (46%; Table 23.1) in the older, basal tissue, where groups of up to four adjacent heterocysts can be found (Costa and Lindblad 2002).

In *Gunnera* glands *Nostoc* heterocyst frequencies of up to 60–80% are observed, whereas levels greater than 10% are not seen in free-living *Nostoc* cultures (Table 23.1; Bergman et al. 2007a; Franche et al. 2009). Nitrogen fixation activity increases in parallel with the increasing heterocyst frequency up to 20% heterocysts after which it declines, despite the continued increase in heterocyst frequency (Bergman 2002). The highest heterocyst frequencies are observed in the oldest symbiotic tissue, 10 mm or more from the stem apex, although *hetR*, the key gene in heterocyst development, is most highly expressed 7–8 mm from the apex (Wang et al. 2004).

In all tripartite lichens the cyanobiont role of N_2 fixation is aided by elevated heterocyst frequencies of 10–55% (Table 23.1) compared with 5–10% in free-living cyanobacteria (Rai 2002; Rai and Bergman 2002; Adams et al. 2006). Again, an increasing trend in heterocyst frequency is found from the youngest to the oldest parts of the thallus. By contrast, the role of the cyanobiont in two-membered cyanolichens is to provide both N and C, which means that elevated heterocyst frequencies (with the resulting decrease in carbon fixation capacity) are not sustainable, so frequencies are similar to those in free-living cyanobacteria (Table 23.1; Rai 2002; Rai and Bergman 2002; Bergman et al. 2007a). Theoretical models of tripartite lichens have confirmed that it is in the interest of the partnership to maximise heterocyst frequency and to maintain a low ratio of cyanobacterial cells to green algal cells (Hyvärinen et al. 2002).

What Regulates Heterocyst Frequency in Plants?

The accuracy of the heterocyst frequencies determined for plant-associated cyanobionts can be questioned because such heterocysts often lose their characteristic regular shape and thickened walls, making them difficult to distinguish from vegetative cells (Meeks and Elhai 2002; Meeks 2003, 2009). In addition, of those heterocysts that can be recognised by light or electron microscopy, at least some are likely to be senescent or dead (Meeks and Elhai 2002). This is supported by the observation that maximum N_2 fixation rates in cyanobionts *in planta* usually occur at intermediate heterocyst frequencies, implying that the multiple contiguous heterocysts found at the highest frequencies include heterocysts that are metabolically inactive. This low metabolic activity may be because they have either reached the end of their natural lifespan, or they are poorly supplied with photosynthate which has to pass from vegetative cells, through the outer heterocysts in a contiguous group, to reach those at the centre of the group. The presence of senescent heterocysts may also at least partly explain the development of multiple contiguous heterocysts, because a new heterocyst may develop next to an existing, but non-functional heterocyst (Meeks and Elhai 2002; Meeks 2009). However, such an explanation is unlikely to apply to the contiguous heterocysts formed in the *Gunnera* isolate *Nostoc* PCC9229 when grown on fructose in the dark (see below; Wouters et al. 2000).

Notwithstanding the above provisos, there is no doubt that heterocyst frequency is greatly elevated in plant symbioses. The question is – how is this controlled? In free-living cyanobacteria elevated heterocyst frequencies can be induced by the immobilisation of filaments in polyurethane or polyvinyl foams and other hollow matrices, or by short-term exposure to increased light intensity or the amino acid analogue 7-azatryptophan (Adams 2000). In the *Gunnera* isolate *Nostoc* PCC9229 three weeks of dark growth in the presence of fructose leads to the development of double and quadruple heterocysts, which are not formed in the absence of fructose (Wouters et al. 2000). These observations raise the possibility that the elevated heterocyst frequencies found in cyanobionts *in planta* may be at least in part due to the environmental conditions they experience.

The signal for heterocyst development in free-living cyanobacteria is starvation for combined nitrogen, which is thought to be perceived via an elevated intracellular concentration of 2-oxoglutarate (Muro-Pastor et al. 2001; Vazquez-Bermudez et al. 2002; Zhang et al. 2006). 2-oxoglutarate activates NtcA, a transcriptional regulator that controls the expression of genes encoding proteins for the uptake and metabolism of N sources other than ammonium (Flores and Herrero 2005; Muro-Pastor et al. 2005). NtcA in turn stimulates transcription of genes encoding both positive (e.g. HetR and HetF) and negative (e.g. PatN and PatS) regulators of heterocyst development (Herrero et al. 2004; Zhang et al. 2006; Meeks 2009).

However, starvation for combined nitrogen is unlikely to be the signal for heterocyst development in symbiotically-associated *Nostoc* because the cyanobiont does not show any of the characteristic features of N limitation, such as the breakdown of N reserves (Sect. 23.6.3). This may imply that the signal for heterocyst differentiation in symbiosis is supplied by the host plant. Support for this theory comes from the behaviour of a *Nostoc punctiforme* mutant defective in the assimilation of nitrate, which as a consequence fails to repress heterocyst development and N_2 fixation (Meeks and Elhai 2002; Meeks 2003). Heterocysts that form in the mutant in the presence of nitrate are defective and incapable of N_2 fixation under oxic conditions, but can fix N_2 within the anoxic slime cavities of the hornwort *Anthoceros*. However, within these slime cavities N_2 fixation is repressed by nitrate, unlike the situation in free-living cultures of the mutant. This repression was shown not to be due to a build-up of ammonium resulting from the reduction of nitrate by *Anthoceros*. These observations imply that *in planta* the regulation of N_2 fixation and heterocyst development is plant-mediated and independent of the N status of the cyanobiont (Meeks 2003, 2009). The molecular target(s) for this plant signal remains to be identified but it seems highly likely that it acts prior to activation of the key heterocyst differentiation gene *hetR* (Zhang et al. 2006) and also prior to *ntcA* (discussed further by Meeks and Elhai 2002; Wong and Meeks 2002; Meeks 2009).

23.6.2.3 Host Changes

Whereas morphological changes are often apparent in symbiotically-associated cyanobacteria, there are generally few obvious post-infection changes in the host. For example, in the *Azolla* association the leaf cavity and the hairs (which are fundamental to the functioning of the association with the cyanobionts) also exist in cyanobiont-free *Azolla* (see: Zheng et al. 2009a), although mucilage production (possibly by all partners in the association) is often regarded as a symbiosis-related change. Those morphological changes that are apparent in hosts often reflect the need for efficient nutrient exchange between the partners, which is essential for a stable symbiosis. Such changes are seen in both *Blasia* and *Anthoceros*; branched, multicellular filaments grow from the walls of the auricle in *Blasia* and the slime cavity in *Anthoceros punctatus*, penetrating the cyanobacterial colony and facilitating nutrient exchange with the host plant (see: Adams 2002a; Adams and Duggan 2008). However, such ingrowths of the cells surrounding the *Nostoc* colonies are not found in many other hornworts, including *Leiosporoceros* (Villarreal and Renzaglia 2006). Once the hornwort slime cavity is colonised the middle lamella between internal cells separates to form an enlarged space,

allowing expansion of the colony; these changes do not occur in uninfected slime cavities (Renzaglia et al. 2000). Similarly, in *Leiosporoceros* the cavities containing the *Nostoc* are rarely seen in the absence of the cyanobacterium (Villarreal and Renzaglia 2006). The *Blasia* auricle also expands once infected by *Nostoc*, enabling the colony to grow (Renzaglia et al. 2000).

In cycads the entry of the cyanobacteria into the coralloid roots triggers significant morphological change, transforming them into typical infected coralloids (see: Costa and Lindblad 2002; Bergman et al. 2007a). Root diameter increases while elongation is reduced and the cells linking the inner and outer cortical layers become elongated, possibly to facilitate the exchange of nutrients between the partners (Costa and Lindblad 2002). These changes create a mucilage-filled space containing tightly-packed cyanobacterial filaments and traversed by elongated host cells which connect the original inner cortical layer and the newly-formed outer layer (Vessey et al. 2005).

In *Gunnera* the cells lining the gland channels divide in response to the presence of compatible cyanobacteria and it is thought that these are the cells that become infected (Bergman et al. 2007a). What induces these changes in *Gunnera* isn't known, although cyanobacteria do produce compounds involved in plant development (Liaimer and Bergman 2004) including the phytohormone auxin, indole-3-acetic acid (Sergeeva et al. 2002). Adaptations specifically for nutrient exchange aren't needed in *Gunnera* because the intracellular location of the cyanobionts ensures efficient transfer of nutrients. Similarly, in the *Geosiphon* symbiosis the location of the cyanobionts within the specialised bladder is sufficient to ensure good nutrient exchange. In lichens, it is the close association between the cyanobiont and thin-walled fungal hyphae, often without contact between fungal and cyanobacterial cell walls, that ensures efficient nutrient exchange (Rikkinen 2002). In a few cases, fungal haustoria penetrate the cyanobiont cell wall and enter the cells.

23.6.3 N_2 Fixation and Transfer of Fixed Nitrogen

In many cyanobacterial symbioses the role of the cyanobiont is to provide combined nitrogen for the host. This is particularly true of plant symbioses in which all known cyanobionts are capable of N_2 fixation (Kneip et al. 2007; Bergman et al. 2007a). By contrast, in the sponge symbioses the cyanobiont role is primarily provision of photosynthate. Nevertheless, although no heterocystous cyanobacteria have been reported as cyanobionts of sponges, there is evidence of N_2 fixation by sponge cyanobionts. Using PCR and RT-PCR, both the presence of and expression of *nifH*, encoding dinitrogenase reductase, one of the two nitrogenase proteins, has been demonstrated in two marine cyanosponges (Mohamed et al. 2008), implying that cyanobacteria may benefit the sponge by provision of fixed N (Taylor et al. 2007). In the ascidian-cyanobacteria symbiosis early reports of N_2 fixation by *Prochloron* have not been confirmed (Carpenter and Foster 2002; Yellowlees et al. 2008). Ammonium is the primary nitrogenous waste of the host ascidian and this may be used by *Prochloron* as a source of N (Kühl and Larkum 2002; Yellowlees et al. 2008).

In many of the plant symbioses, such as the cycads, *Gunnera* and *Azolla*, the gradient of heterocyst frequency, from low in the youngest tissue to high in the oldest tissue is paralleled by a gradient of low to high nitrogenase activity, although the highest rate of N_2 fixation is often at intermediate heterocyst frequencies (Meeks and Elhai 2002; Meeks 2003, 2009; Bergman et al. 2007a). Such a gradient of N_2 fixing capacity is particularly apparent in the stolon of *Gunnera magellanica*, in which the lowest rate is found in newly-infected tissue and the highest in the region of the stolon where the heterocyst frequency is 20%. A corresponding increase in expression of genes involved in heterocyst development (*hetR* and *ntcA*) and nitrogenase (*nifH*) is also apparent (Wang et al. 2004) although it isn't known if these are the results of direct control by the plant, or are a consequence of the conditions experienced by the cyanobiont *in planta*. As heterocyst frequency continues to increase to 60% further along the stolon, the N_2 fixation rate declines (Bergman 2002; Bergman et al. 2007a). This decline in rate may result from poor transfer of photosynthate into heterocysts, particularly within the groups of adjacent heterocysts found in the regions of highest heterocyst frequency. This is probably because the cell envelopes of heterocysts are impermeable to gases and solutes (Walsby 2007) and so photosynthate must pass to the internal heterocysts of an adjacent group via the outer heterocysts.

In the *Azolla* symbiosis a gradient of increasing nitrogenase activity coincides with the increase in heterocyst frequency in the younger leaves along the main axis of the plant (see: Meeks and Elhai 2002; Bergman et al. 2007a; Meeks 2009). Nitrogenase is oxygen-labile and is protected in part by the thickened cell walls of the heterocyst and is thereby immune to the oxygen concentrations in the *Azolla* leaf cavity (Lechno-Yossef and Nierzwicki-Bauer 2002; Meeks et al. 2002). Oxygen concentration is lower in the midsection (where nitrogenase activity is high) compared with the apex and the base of the plant. Respiration of the whole symbiosis system probably accounts for the lowering of the oxygen concentration and might also be a valuable source of ATP for the reduction of molecular nitrogen (Lechno-Yossef and Nierzwicki-Bauer 2002), supported by elevated levels of ATP synthase and ferredoxin $NADP^+$ reductase in the cyanobiont (Ekman et al. 2008).

Nitrogen fixation in the endosymbiotic cyanobiont (the so-called spheroid body) of the diatom *Rhopalodia gibba* is strictly light dependent (Kneip et al. 2007) yet the closest relative of the cyanobiont is the unicellular N_2 fixing cyanobacterium *Cyanothece* which protects nitrogenase from oxygen inactivation by temporal separation of N_2 fixation (at night) and photosynthesis (by day). Light-dependent N_2 fixation in the diatom cyanobiont is possible because the cyanobacterium has lost the capacity for photosynthesis (and hence oxygen production) and instead is supplied with fixed carbon by the diatom. The symbionts in dinoflagellate *Histioneis depressa* label positively with anti-nitrogenase antibodies, so may be capable of N_2 fixation (Foster et al. 2006a).

23.6.3.1 Release of N to the Host

In many cyanobacterial associations much of the N_2 fixed by the cyanobiont is released to the host, the proportion retained by the symbiont varying considerably (Table 23.1) from as much as 50% in *Azolla* to as little as 10–20% in lichens, *Gunnera* and hornworts (Meeks and Elhai 2002; Rai 2002; Meeks 2003, 2009; Bergman et al. 2007a). Ammonia is the form of N released from the cyanobionts in liverworts, hornworts, *Azolla* and lichens (Rai 2002; Bergman et al. 2007a), ammonia and some asparagine in *Gunnera* (Bergman 2002; Bergman et al. 2007a), whereas in cycads the form is thought to be glutamine and possibly citrulline (Table 23.1; Costa and Lindblad 2002; Vessey et al. 2005).

Ammonia release can often be at least partly explained by decreased activity of glutamine synthetase (GS) in the cyanobiont (although in the cycad symbiosis this seems not to be the case; see below); this enzyme is part of the glutamine synthetase-glutamate synthase (GS-GOGAT) pathway, which is the primary route of ammonia assimilation in cyanobacteria (Muro-Pastor et al. 2005; Flores and Herrero 2005). The decrease in GS activity in most symbiotically-associated cyanobacteria is achieved by a reduction in either the activity of the enzyme or in the amount of protein produced, and this varies in different hosts (Table 23.1). In hornworts, and presumably also liverworts, there is a reduction in the activity of GS (Meeks and Elhai 2002; Meeks 2003, 2009). The mechanism of this reduction isn't known, but it is likely to involve post-translational modification of the enzyme because there is little difference in the level of GS protein between *Anthoceros*-associated *Noctoc* and free-living cyanobacteria (Table 23.1; Meeks and Elhai 2002; Meeks 2003, 2009).

By contrast, the 70% reduction in GS activity in the *Azolla* cyanobiont can be accounted for by the very low levels of GS protein (Table 23.1), which in the *A. caroliniana* cyanobiont are 5–40% of those in free-living *Nostoc* and *Anabaena* strains (Rai et al. 2000; Pabby et al. 2004a; Meeks 2009). In addition, low levels of *glnA* (encoding GS) transcripts (10% of the levels found in the free-living cyanobacteria) have been reported, leading to the suggestion that a host-derived factor(s) selectively represses *glnA* expression in the cyanobiont (most recently discussed by Meeks 2009). Immunogold electron microscopy has revealed that GS concentration in the heterocysts of the *Azolla* cyanobiont is decreased to that normally associated with vegetative cells (Rai et al. 2000; Meeks 2009). More recently Ekman et al. (2008) also reported a reduction in the amount of GS protein in the *A. caroliniana* cyanobiont compared with a free-living *Nostoc* species. In summary, repression and/or lack of stimulation of GS synthesis (and possibly also the inactivation or inhibition of the remaining enzyme) limits ammonium assimilation in symbiotically-associated heterocysts, consequently leading to the release of ammonium into the *Azolla* leaf cavity, presumably via the vegetative cells as the heterocyst cell envelope is impermeable to gases and solutes (Walsby 2007).

In lichen cyanobionts the release of ammonia is a consequence of a reduction in the activity of both GS and GOGAT, the former by up to 90% as a result of a reduction in GS synthesis and hence the amount of the enzyme (Table 23.1; Rai 2002; Rai and Bergman 2002; Adams et al. 2006). By contrast, in the *Gunnera* cyanobiont the amount of GS protein is unchanged in symbiosis, but its specific activity is reduced to 70% of that in the free-living state (Bergman et al. 2007a; Table 23.1). Cycad cyanobionts release organic N (possibly glutamine and citrulline) rather than ammonia and have GS and GOGAT activities similar to those in free-living cyanobacteria (Costa and Lindblad 2002; Lindblad and Costa 2002; see below and Table 23.1).

Despite releasing much of the nitrogen they fix, cyanobionts do not show the physiological signs of nitrogen starvation, because they display characteristics associated with N excess, such as the presence of carboxysomes, cyanophycin granules and phycobilisomes which, under nitrogen deprivation, would be degraded to provide amino acids for protein synthesis (Meeks and Elhai 2002). Phycobiliproteins are both accessory photopigments and serve as a nitrogen reserve to be used during N starvation. Cyanophycin is a specialised N reserve consisting of a co-polymer of arginine and aspartic acid. Both of these are found in the cyanobionts of many plants hosts. For example, in the *Azolla* cyanobiont the percentage of vegetative cells containing cyanophycin is low in the apex (45%) and at the base of the axis (60%) but higher in the mid-section of the plant (80–85%) where heterocyst frequency and N_2 fixation rate are also higher (Lechno-Yossef and Nierzwicki-Bauer 2002; Zheng et al. 2009a).

23.6.3.2 Host Uptake of N

After release from the cyanobiont, the ammonia is taken up the GS-GOGAT pathway of the host in the case of bryophytes and *Azolla*, or by glutamate dehydrogenase in the case of the fungus in cyanolichens (Rai 2002; Bergman et al. 2007a). Plants have two types of GS isoenzymes, GS1 found in

the cytosol and GS2 that localises in plastids/chloroplasts. Immunogold labelling using an anti-*Azolla* GS2 antibody has shown that the *Azolla* GS occurs not only in chloroplasts but also in the cytoplasm of hair cells (Uheda et al. 2004). Moreover GS synthesis in the hair cells is specifically stimulated by the presence of the ammonium fixed and released by the cyanobiont. By contrast, in cyanobiont-free plants labelling of GS in hair cells is very weak (Uheda et al. 2004; Uheda and Maejima 2009).

The fixed nitrogen must next be transported throughout the host tissues. The major forms of N moving from the *Azolla* leaf cavity to the stem apex are thought to be glutamate (and possibly a glutamate derivative), glutamine and ammonia (Rai et al. 2000), whereas in cycads it is glutamine and citrulline, or in some cycad groups glutamine and glutamic acid, that are thought to be transported from roots to stem *via* the xylem (Costa and Lindblad 2002: Bergman et al. 2007a). Alanine is thought to be the form in which N moves from the cephalodia to the main thallus in tripartite lichens (Rai 2002; Bergman et al. 2007a). In *Gunnera monoica* fixed nitrogen is transported via the phloem (rather than the xylem as in other angiosperm-bacteria symbioses) from mature regions of the plant to apical regions and the leaves (Bergman et al. 2007a).

23.6.4 CO_2 Assimilation and Transfer of Carbon

In many cyanobacteria symbiotically associated with a photosynthetic host the rate of light-dependent CO_2 fixation is greatly reduced from the free-living state and the cyanobiont grows photo- or chemo-heterotrophically using fixed carbon supplied by its host. The primary route for the fixation of CO_2 in cyanobacteria is the Calvin-Benson-Bassham cycle, and ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) is the primary carboxylating enzyme. A reduction in the specific activity of this enzyme, by an unknown mechanism, is responsible for the reduction of CO_2 fixation in some, but not all, plant symbioses (Table 23.2 and see below). The mechanism of inhibition may well vary in different symbioses.

Immediately following isolation from *Anthoceros* the *Nostoc* symbiont shows only 12% of the light-dependent CO_2 fixation of the free-living cyanobacterium (Table 23.2). This reduced activity seems to be the result of a post-translational modification of the Rubisco protein, as the amount of protein differs little between free-living or *Anthoceros*-associated *Nostoc* (Meeks and Elhai 2002; Meeks 2003). Because of its low CO_2 fixation capacity the *Nostoc* cyanobiont requires a supply of sugars from the host (Meeks and Elhai 2002; Meeks 2003).

The cyanobionts of cycads receive little if any light and are assumed to receive their carbon from the host, but the details are unknown (Costa and Lindblad 2002). Despite their heterotrophic nutrition and lack of CO_2 fixation, the cyanobionts retain Rubisco, which is active in extracts of the cyanobacteria freshly-isolated from the cycad (Table 23.2).

Table 23.2 Characteristics of light-dependent CO_2 fixation and ribulose bisphosphate carboxylase in symbiotically-associated cyanobacteria (Table compiled from Meeks (2009) and Adams (2000) and references therein)

Host	Light-dependent CO_2 fixation (%)	Rubisco	
		Protein (%)	Specific activity (%)
Cycads	0	ND	87–100
Gunnera	<2	100	100
Hornwort (*Anthoceros punctatus*)	12	100	12–15
Azolla	85–100	ND	ND
Lichens:			
Bipartite	ND	~100	ND
Tripartite	~8	~100	~8

Percentages indicate the value in the cyanobiont immediately after isolation from the host, compared with that in the free-living strain
ND not determined

In the *Gunnera-Nostoc* symbiosis it appears that rather than a total loss of the cyanobiont cellular photosynthetic machinery, there are modifications of key components (such as the D1 protein of the photosystem II complex) to render the pathway inactive (Black and Osborne 2004). This shift signals a change from a photoautotrophic to a heterotrophic metabolic state, perhaps triggered by the presence of fixed carbon from the plant, possibly in the form of glucose or fructose (Black et al. 2002; Black and Osborne 2004). Indeed, fructose and glucose support dark N_2 fixation in the free-living *Gunnera* isolate *Nostoc* PCC 9229 and after 4 weeks dark growth on fructose multiple adjacent heterocysts are found, together with high expression of *hetR*, reminiscent of the symbiotic growth state (Wouters et al. 2000). In addition, *Nostoc* PCC 9229 produces a putative cyclodextrin glucosyl transferase in the light and dark when fructose is supplied (Wouters et al. 2003). This enzyme is a member of the α-amylase family and typically catalyses the hydrolysis of, for example, α-D-glucose.

In *Azolla*, photosynthetic activity and CO_2 fixation by the cyanobiont are significantly reduced, contributing less than 5% of the photosynthetic oxygen evolution and CO_2 fixed in the association (Meeks 2009). Transcripts for Rubisco are also some 5–7-fold lower in the cyanobiont compared with free-living cultures (see: Adams 2000; Rai et al. 2000; Meeks 2009). Similarly, proteomic analysis has revealed lower levels of Rubisco in the *Azolla* cyanobiont than in cultured *Nostoc* PCC 73102 (Ekman et al. 2008). Surprisingly, immediately after isolation from the plant, the primary cyanobiont has approximately 85% of the photosynthetic rate of free-living cyanobacteria (Table 23.2); why this should be is not known. Energy requirements of the cyanobiont are believed

to be met in part by carbohydrates, mainly sucrose or possibly fructose, supplied by the host (Ekman et al. 2006). Correspondingly, Ekman et al. (2006) revealed an up-regulation in key enzymes potentially involved in the assimilation of host-derived carbon sources, including a likely hexose transporter that was four times more abundant in the *Azolla* cyanobiont than its homologue in the free-living *Nostoc* strain. The oxidative pentose phosphate pathway (OPP) is the major route of carbon catabolism in cyanobacteria and is a source of reductant to both nitrogenase and oxidative respiration. Elevated levels of the OPP enzyme 6-phosphogluconate dehydrogenase in the *Azolla* cyanobiont may be a reflection of the higher demands for reductant during symbiotic growth (Ekman et al. 2008).

Cyanobionts of bipartite lichens transfer 70–80% of the carbon they fix, in the form of glucose, to the fungal host, whereas in tripartite lichens the cyanobiont transfers little, if any, fixed carbon to the mycobiont (Palmqvist 2000, 2002; Adams et al. 2006; Bergman et al. 2007a). The transferred glucose is rapidly converted to mannitol, which can only be used by the mycobiont. In tripartite lichens cyanobionts in cephalodia located within the cortex or underneath the thallus may receive so little light that they are not photosynthetically active and so must receive carbon from the primary photobiont (Palmqvist 2002; Bergman et al. 2007a). The rate of CO_2 diffusion is 10,000 times lower in water than in air, and so the hydration state of the thallus greatly affects photosynthesis (Palmqvist 2002). This is because a damp thallus still has air spaces through which CO_2 can diffuse rapidly, whereas these spaces are filled with water in a wet thallus and swelling of the fungal hyphae can also block gaseous pores. Cyanolichens can be at an advantage in wet conditions because they have a CO_2-concentrating mechanism that can at least partly compensate for the reduced CO_2 diffusion (Palmqvist 2002). However, even cyanolichens vary in their ability to photosynthesise under very wet conditions (Lange et al. 2004).

In the *Geosiphon* symbiosis the function of the *Nostoc* is primarily photosynthesis and accordingly its photosynthetic activity is greater than in the free-living state (Kluge 2002; Adams et al. 2006). Carbon is transferred from *Nostoc* to the fungal host possibly in the form of sucrose, although it has been suggested that degradation of *Nostoc* extracellular polysaccharides may release hexoses that could be transported into the fungus by the hexose transporter GpMST1 found in *Geosiphon pyriformis* (Shüssler et al. 2006)

In the remaining cyanobacterial symbioses the details of symbiont CO_2 fixation and transfer of carbon to the host are poorly understood. Cells of the *Prochloron* cyanobiont of ascidians contain ribulose bisphosphate carboxylase in carboxysomes and CO_2 uptake is aided by carbonic anhydrase activity (Griffiths 2006; Yellowlees et al. 2008). Carbon photosynthetically fixed by *Prochloron* can be transferred to the host and, in *Didemnum molle* and *Lissoclinum patella*, may provide between 12% and 56% of its reduced carbon requirement (Griffiths 2006; Yellowlees et al. 2008). In the marine diatom *Rhizosolenia* the cyanobiont, *Richelia intracellularis*, provides its host with both N and C (Adams 2000). By contrast the unicellular cyanobionts of the dinoflagellates *Ornithocerus magnificus* and *Ornithocerus steinii*, appear to provide only fixed carbon for their non-photosynthetic host. In the case of sponge-cyanobacteria symbioses carbon transferred from the cyanobiont to the sponge, possibly as glycerol and organic phosphate, can satisfy up to 50% of the host's energy requirement and 80% of its carbon budget (Erwin and Thacker 2007; Usher 2008).

23.7 Artificial Cyanobacteria-Plant Symbioses

There have been numerous attempts to construct novel associations between plants and cyanobacteria, the goal being the replacement of at least a proportion of current artificial nitrogenous fertiliser used for crop plants, with the resultant commercial and environmental benefits. This work has so far involved the introduction of cyanobacteria into higher plant protoplasts (see: Rai et al. 2000; Adams 2000; Gusev et al. 2002; Bergman et al. 2007a), or the co-culture of cyanobacteria with plant tissue cell cultures, plant regenerates and cuttings from a variety of plants (Gantar 2000b; Gorelova 2001, 2006; Lobakova et al. 2001a, b; Gorelova and Korzhenevskaya 2002; Gorelova and Kleimenov 2003; Gorelova and Baulina 2009; see also Rai et al. 2000 and Gusev et al. 2002 for a discussion of the earlier literature). Some *Nostoc* strains have been shown to be attracted to exudates from plants that do not normally serve as hosts (Nilsson et al. 2006) and to colonise the surface of the roots of rice (Nilsson et al. 2002; 2005) and wheat (Karthikeyan et al. 2007, 2009; Sood et al. 2011). Mechanical damage of wheat seedling roots can result in growth of cyanobacteria within the root tissues (Gantar 2000a) and in laboratory tests the colonization of wheat roots by some heterocystous cyanobacteria can lead to enhancement of plant N content and root growth (for a discussion of this see: Rai et al. 2000; Adams 2000; Gusev et al. 2002; Bergman et al. 2007a).

23.8 Concluding Remarks

This chapter has discussed the many known cyanobacterial symbioses, but where might there be new ones waiting to be discovered? Novel cyanobacteria-plant associations are perhaps most likely to occur where the conditions for the survival of hormogonia are optimum. For the infection of plant roots this may be in almost any soil, but for the infection of stems or leaves this is likely to require a good level of

moisture, such as that found in temperate and tropical rain forests and in wet boreal forests where mosses thrive. These moist environments are also ideal for the survival of epiphytic cyanobacteria which may supply combined nitrogen to the plant itself or to the local ecosystem, as is the case for the cyanobacteria-moss associations. Perhaps in such wet and humid environments might be found a modern-day higher plant equivalent of the extinct *Aglaophyton major-Archaeothrix* symbiosis in which hormogonia infected the leaves via stomatal pores in a manner similar to the extant hornwort *Leiosporoceros dussii*. The oceans are also likely to be a major source of undiscovered cyanobacterial symbioses, an interesting potential example of which is the marine, N_2 fixing unicellular cyanobacterium UCYN-A (Zehr et al. 2008; Tripp et al. 2010) which is evolutionarily related to the spheroid bodies of *Rhopalodia gibba* (Bothe et al. 2010). This cyanobacterium lacks the photosystem II complex and the biosynthetic pathways for several amino acids and purines, implying that it is reliant on a symbiotic partner, although such a partner has yet to be identified.

Another question to consider is the potential for the future agricultural use of cyanobacteria-plant symbioses. Only *Azolla* has been used as a green manure in agriculture. However, N only becomes available to the rice plants when *Azolla* decays. A more efficient delivery of combined nitrogen to a crop would require the growth of cyanobacteria on the surface of, or within the plant. Such symbioses do not exist, so what is the likelihood of creating them artificially? As N_2 fixing symbionts, cyanobacteria such as *Nostoc* spp. have the great advantage that they possess, in the heterocyst, a unique system for the protection of nitrogenase from oxygen inactivation. They also show a catholic taste in hosts and have a highly developed infective agent, the hormogonium. *Nostoc* spp. would therefore make ideal N_2 fixing symbionts for the creation of novel symbioses with crop plants. To avoid the need to re-establish the partnership at each generation these symbioses would ideally be self-perpetuating. Yet, despite the (presumed) long evolutionary history of cyanobacteria-plant symbioses, *Azolla* is the only example in which the cyanobiont is passed from one generation to the next. It seems likely therefore, that the chance of creating novel, self-perpetuating cyanobacterial endosymbioses with crop plants, is not high (Bergman et al. 2007a). In addition, because the cyanobiont in all cyanobacteria-plant symbioses occupies existing structures (although these can undergo modification following infection) plants lacking suitable structures will not provide ready hosts. A further problem is that modern cereal crop varieties have been bred for rapid growth, requiring high rates of N input for short periods, and cyanobacteria are likely to provide at best only a portion of the N required (Bergman et al. 2007a). The introduction of cyanobacteria to a slower-growing crop plant may therefore be most effective at fulfilling the N requirements of the plant.

In the laboratory, *Nostoc* spp. have been shown to colonise the roots of wheat seedlings and enhance root and plant growth. It isn't known if such interactions occur in the field, but if they do, then a relatively simple strategy would be to enhance these existing interactions by producing plant varieties that release larger amounts of the chemical signals that induce and attract hormogonia, thereby stimulating colonization of roots to the benefit of the plant. What is lacking at present is knowledge of the chemical identity of these plant signals. This approach would have the benefit of not requiring the introduction of mutant cyanobacteria into the field where they would be unlikely to compete with natural populations.

Another stumbling block to the creation of artificial cyanobacteria-plant symbioses is our poor understanding of the involvement of the plant partner in existing symbioses. Most research effort has focussed on the cyanobiont, with relatively little attention given to the plant, perhaps because such work is more technically difficult. We need a much clearer understanding of the signals exchanged by the partners, because the ability to produce and respond to these signals will be vital to the chances of any cyanobiont forming a stable symbiosis with a potential plant host. Indeed, the scarcity of plant cyanobionts from some heterocystous- and hormogonia-producing genera may be a result of their inability to respond appropriately to plant signals.

Finally, what is the environmental impact of cyanobacterial symbioses? It is becoming clear that many, by virtue of their capacity for N_2 fixation, are of major environmental significance. Many of the known marine cyanobacterial symbioses, such as those with diatoms and dinoflagellates, are poorly understood, yet their abundance and N_2 fixing capacity can have a major impact on N availability in the open oceans. On a smaller scale, benthic N_2 fixing cyanobacterial symbioses with sponges and sea grasses can supply significant amounts of N to their local ecosystems. The impact of N_2 fixing cyanobacterial symbioses is of course greatest where alternative N inputs are least; so, cyanolichens and cyanobacteria-moss associations can have major impacts in many regions of the northern hemisphere where inputs of combined nitrogen from N_2-fixing plants and atmospheric deposition are low. Of course future climate change may have significant impacts on these symbioses and the ecosystems that their N_2 fixation supports.

References

Adams DG (2000) Symbiotic interactions. In: Whitton BA, Potts M (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 523–561, 669 pp

Adams DG (2002a) Cyanobacteria in symbiosis with hornworts and liverworts. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 117–135

Adams DG (2002b) The liverwort-cyanobacterial symbiosis. Biol Environ Proc R Ir Acad 102B:27–30
Adams DG, Duggan PS (1999) Heterocyst and akinete differentiation in cyanobacteria. New Phytol 144:3–33
Adams DG, Duggan PS (2008) Cyanobacteria-bryophyte symbioses. J Exp Bot 59:1047–1058
Adams DG, Bergman B, Nierzwicki-Bauer SA, Rai AN, Schüßler A (2006) Cyanobacterial-plant symbioses. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The prokaryotes. A handbook on the biology of bacteria, vol 1, 3rd edn, Symbiotic associations, biotechnology, applied microbiology. Springer, New York, pp 331–363
Aguiar R, Fiore MF, Franco MW, Ventrella MC, Lorenzi AS, Vanetti CA, Alfenas AC (2008) A novel epiphytic cyanobacterial species from the genus *Brasilonema* causing damage to eucalyptus leaves. J Phycol 44:1322–1334
Antoine ME (2004) An ecophysiological approach to quantifying nitrogen fixation by *Lobaria oregana*. Bryologist 107:82–87
Ariosa Y, Quesada A, Aburto J, Carrasco D, Carreres R, Leganes F, Valiente EF (2004) Epiphytic cyanobacteria on *Chara vulgaris* are the main contributors to N_2 fixation in rice fields. Appl Environ Microbiol 70:5391–5397
Armitage AR, Frankovich TA, Fourqurean JW (2006) Variable responses within epiphytic and benthic microalgal communities to nutrient enrichment. Hydrobiologia 569:423–435
Arrigo KR (2005) Marine microorganisms and global nutrient cycles. Nature 437:349–355
Asplund J, Gauslaa Y (2010) The gastropod *Arion fuscus* prefers cyanobacterial to green algal parts of the tripartite lichen *Nephroma arcticum* due to low chemical defence. Lichenologist 42:113–117
Badger MR, Price GD, Long BM, Woodger FJ (2006) The environmental plasticity and ecological genomics of the cyanobacterial CO_2 concentrating mechanism. J Exp Bot 57:249–265
Baker JA, Entsch B, McKay DB (2003) The cyanobiont in an *Azolla* fern is neither *Anabaena* nor *Nostoc*. FEMS Microbiol Lett 229:43–47
Banack SA, Murch SJ, Cox PA (2006) Neurotoxic flying foxes as dietary items for the Chamorro people, Marianas Islands. J Ethnopharmacol 106:97–104
Banack SA, Johnson HE, Cheng R, Cox PA (2007) Production of the neurotoxin BMAA by a marine cyanobacterium. Mar Drugs 5:180–196
Bar Zeev E, Yogev T, Man-Aharonovich D, Kress N, Herut B, Beja O, Berman-Frank I (2008) Seasonal dynamics of the endosymbiotic, nitrogen-fixing cyanobacterium *Richelia intracellularis* in the eastern Mediterranean Sea. ISME J 2:911–923
Bates ST, Cropsey GWG, Caporaso JG, Knight R, Fierer N, Fierer N (2011) Bacterial communities associated with the lichen symbiosis. Appl Environ Microbiol 77:1309–1314
Baulina OI, Lobakova ES (2003a) Atypical cell forms overproducing extracellular substances in populations of cycad cyanobionts. Microbiology 72:701–712
Baulina OI, Lobakova ES (2003b) Heterocysts with reduced cell walls in populations of cycad cyanobionts. Microbiology 72:713–722
Becerro MA, Turon X, Uriz MJ, Templado J (2003) Can a sponge feeder be a herbivore? *Tylodina perversa* (Gastropoda) feeding on *Aplysina aerophoba* (Demospongiae). Biol J Linn Soc 78:429–438
Belnap J (2001) Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In: Belnap J, Lange OL (eds) Biological soil crusts: structure, function and management. Springer, New York, pp 241–261
Belnap J (2002) Nitrogen fixation in biological soil crusts from Southeast Utah, USA. Biol Fertil Soils 35:128–135
Benner JW, Vitousek PM (2007) Development of a diverse epiphyte community in response to phosphorus fertilization. Ecol Lett 10:628–636
Benner JW, Conroy S, Lunch CK, Toyoda N, Vitousek PM (2007) Phosphorus fertilization increases the abundance and nitrogenase activity of the cyanolichen *Pseudocyphellaria crocata* in Hawaiian montane forests. Biotropica 39:400–405
Bennicelli R, Stepniewska Z, Banach A, Szajnocha K, Ostrowski J (2004) The ability of *Azolla caroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. Chemosphere 55:141–146
Benson J, Margulis L (2002) The *Gunnera manicata-Nostoc* symbiosis: is the red stipulate tissue symbiogenetic? Biol Environ Proc R Ir Acad 102B:45–48
Bergman B (2001) Nitrogen-fixing cyanobacteria in tropical oceans, with emphasis on the Western Indian Ocean. S Afr J Bot 67:426–432
Bergman B (2002) The *Nostoc-Gunnera* symbiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 207–232
Bergman B, Osborne B (2002) The *Gunnera-Nostoc* symbiosis. Biol Environ Proc R Ir Acad 102B:35–39
Bergman B, Johansson C, Söderbäck E (1992) The *Nostoc-Gunnera* symbiosis. New Phytol 122:379–400
Bergman B, Rasmussen U, Rai AN (2007a) Cyanobacterial associations. In: Elmerich C, Newton WE (eds) Associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations. Kluwer Academic Publishers, Dordrecht, pp 257–301
Bergman B, Zheng W, Ekman M, Ran L (2007b) The cyanobacterium--*Azolla* symbiosis: interactions and cell differentiation. Comp Biochem Physiol A Mol Integr Physiol 146A:S218
Bergman B, Ran L, Adams DG (2008a) Cyanobacterial-plant symbioses: signaling and development. In: Herrero A, Flores E (eds) The cyanobacteria: molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 447–473
Bergman B, Zheng WW, Klint J, Ran L (2008b) On the origin of plants and relations to contemporary cyanobacterial-plant symbioses. Plant Biotechnol 25:213–220
Berry AM, Rasmussen U, Bateman K, Huss-Danell K, Lindwall S, Bergman B (2002) Arabinogalactan proteins are expressed at the symbiotic interface in root nodules of *Alnus* spp. New Phytol 155:469–479
Bhaya D (2004) Light matters: phototaxis and signal transduction in unicellular cyanobacteria. Mol Microbiol 53:745–754
Bjerke JW, Galloway DJ, Elvebakk A, Quilhot W (2003a) *Pseudocyphellaria dasyphyllidia* – a new phyllidiate species from Chile (Lobariaceae, lichenised Ascomycota). Cryptogam Mycol 24:59–66
Bjerke JW, Zielke M, Solheim B (2003b) Long-term impacts of simulated climatic change on secondary metabolism, thallus structure and nitrogen fixation activity in two cyanolichens from the Arctic. New Phytol 159:361–367
Björn LO (2007) Stratospheric ozone, ultraviolet radiation, and cryptogams. Biol Conserv 135:326–333
Black K, Osborne B (2004) An assessment of photosynthetic downregulation in cyanobacteria from the *Gunnera-Nostoc* symbiosis. New Phytol 162:125–132
Black KG, Parsons R, Osborne BA (2002) Uptake and metabolism of glucose in the *Nostoc-Gunnera* symbiosis. New Phytol 153:297–305
Bombar D, Moisander PH, Dippner JW, Foster RA, Voss M, Karfeld B, Zehr JP (2011) Distribution of diazotrophic microorganisms and *nifH* gene expression in the Mekong River plume during intermonsoon. Mar Ecol Prog Ser 424:39–55
Bond-Lamberty B, Gower ST (2007) Estimation of stand level leaf area for boreal bryophytes. Oecologia 151:584–592
Bothe H, Tripp JH, Zehr JP (2010) Unicellular cyanobacteria with a new mode of life: the lack of photosynthetic oxygen evolution allows nitrogen fixation to proceed. Arch Microbiol 192:783–790
Botting RS, Campbell J, Fredeen AL (2008) Contrasting arboreal and terrestrial macrolichen and bryophyte communities in old-growth sub-boreal spruce forests of central British Columbia. Bryologist 111:607–619

Bowker MA, Belnap J, Davidson DW (2010) Microclimate and propagule availability are equally important for rehabilitation of dryland N-fixing lichens. Restor Ecol 18:30–33

Brehm U, Krumbein WE, Paliniska KA (2003) Microbial spheres: a novel cyanobacterial-diatom symbiosis. Naturwissenschaften 90:136–140

Brehm U, Krumbein WE, Palinska KA (2006) Biomicrospheres generate ooids in the laboratory. Geomicrobiol J 23:545–550

Brenner ED, Stevenson DW, Twigg RW (2003) Cycads: evolutionary innovations and the role of plant-derived neurotoxins. Trends Plant Sci 8:446–452

Brown PJ, Dalton DA (2002) *In situ* physiological monitoring of *Lobaria oregana* transplants in an old-growth forest canopy. Northwest Sci 76:230–239

Brümmer F, Pfannkuchen M, Baltz A, Hauser T, Thiel V (2008) Light inside sponges. J Exp Mar Biol Ecol 367:61–64

Büdel B, Meyer A, Salazar N, Zellner H, Zotz G, Lange OL (2000) Macrolichens of montane rain forests in Panama, Province Chiriqui. Lichenologist 32:539–551

Büdel B, Darienko T, Deutschewitz K, Dojani S, Friedl T, Mohr KI, Salisch M, Reisser W, Weber B (2009) Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. Microbial Ecol 57:229–247

Bürja AM, Hill RT (2001) Microbial symbionts of the Australian Great Barrier Reef sponge, *Candidaspongia flabellata*. Hydrobiologia 461:41–47

Burns BP, Goh F, Allen M, Neilan BA (2004) Microbial diversity of extant stromatolites in the hypersaline marine environment of Shark Bay, Australia. Environ Microbiol 6:1096–1101

Burrows LL (2005) Weapons of mass retraction. Mol Microbiol 57:878–888

Caldiz MS, Brunet J, Nihlgard B (2007) Lichen litter decomposition in *Nothofagus* forest of northern Patagonia: biomass and chemical changes over time. Bryologist 110:266–273

Campbell J, Fredeen AL (2007) Contrasting the abundance, nitrogen and carbon of epiphytic macrolichen species between host trees and soil types in a sub-boreal forest. Can J Bot 85:31–42

Campbell EL, Wong FCY, Meeks JC (2003) DNA binding properties of the HrmR protein of *Nostoc punctiforme* responsible for transcriptional regulation of genes involved in the differentiation of hormogonia. Mol Microbiol 47:573–582

Campbell EL, Summers ML, Christman H, Martin ME, Meeks JC (2007) Global gene expression patterns of *Nostoc punctiforme* in steady state dinitrogen-grown heterocyst-containing cultures, and at single time points during the differentiation of akinetes and hormogonia. J Bacteriol 189:5247–5256

Campbell EL, Christman H, Meeks JC (2008) DNA microarray comparisons of plant factor- and nitrogen deprivation-induced hormogonia reveal decision-making transcriptional regulation patterns in *Nostoc punctiforme*. J Bacteriol 190:7382–7391

Carpenter EJ (2002) Marine cyanobacterial symbioses. Biol Environ Proc R Ir Acad 102B:15–18

Carpenter EJ, Foster RA (2002) Marine cyanobacterial symbioses. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 11–17

Carpenter EJ, Janson S (2000) Intracellular cyanobacterial symbionts in the marine diatom *Climacodium frauenfeldianum* (Bacillariophyceae). J Phycol 36:540–544

Casamatta DA, Gómez SR, Johansen JR (2006) *Rexia erecta* gen. et sp. nov. and *Capsosira lowei* sp nov., two newly described cyanobacterial taxa from the Great Smoky Mountains National Park (USA). Hydrobiologia 561:13–26

Cebrian E, Uriz MJ, Garrabou J, Ballesteros E (2011) Sponge mass mortalities in a warming Mediterranean sea: are cyanobacteria-harboring species worse off? PLoS One 6:e20211

Chapman KE, Duggan PS, Billington NA, Adams DG (2008) Mutation at different sites in the *Nostoc punctiforme cyaC* gene, encoding the multiple-domain enzyme adenylate cyclase, results in different levels of infection of the host plant *Blasia pusilla*. J Bacteriol 190:1843–1847

Chen CP, Tseng CH, Chen CA, Tang SL (2011) The dynamics of microbial partnerships in the coral *Isopora palifera*. ISME J 5:728–740

Chiu WL, Peters GA, Levieille G, Still PC, Cousins S, Osborne B, Elhai J (2005) Nitrogen deprivation stimulates symbiotic gland development in *Gunnera manicata*. Plant Physiol 139:224–230

Choudhury A, Kennedy IR (2004) Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biol Fertil Soils 39:219–227

Cohen MF, Yamasaki H (2000) Flavonoid-induced expression of a symbiosis-related gene in the cyanobacterium *Nostoc punctiforme*. J Bacteriol 182:4644–4646

Cohen MF, Sakihama Y, Takagi YC, Ichiba T, Yamasaki H (2002) Synergistic effect of deoxyanthocyanins from symbiotic fern *Azolla* spp. on *hrmA* gene induction in the cyanobacterium *Nostoc punctiforme*. Mol Plant Microbe Interact 15:875–882

Cornelissen JHC, Callaghan TV, Alatalo JM, Michelsen A, Graglia E, Hartley AE, Hik DS, Hobbie SE, Press MC, Robinson CH, Henry GHR, Shaver GR, Phoenix GK, Gwynn-Jones D, Jonasson S, Chapin FS III, Molau U, Neill C, Lee JA, Melillo JM, Sveinbjornsson B, Aerts R (2001) Global change and arctic ecosystems: is lichen decline a function of increases in vascular plant biomass? J Ecol 89:984–994

Cornelissen JHC, Lang SI, Soudzilovskaia NA, During HJ (2007) Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. Ann Bot 99:987–1001

Costa JL, Lindblad P (2002) Cyanobacteria in symbiosis with cycads. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 195–205

Costa JL, Paulsrud P, Rikkinen J, Lindblad P (2001) Genetic diversity of *Nostoc* symbionts endophytically associated with two bryophyte species. Appl Environ Microbiol 67:4393–4396

Costa JL, Paulsrud P, Lindblad P (2002) The cyanobacterial $tRNA^{Leu}$ (UAA) intron: evolutionary patterns in a genetic marker. Mol Biol Evol 19:850–857

Costa JL, Romero EM, Lindblad P (2004) Sequence based data supports a single *Nostoc* strain in individual coralloid roots of cycads. FEMS Microbiol Ecol 49:481–487

Cox PA, Banack SA, Murch SJ (2003) Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc Natl Acad Sci USA 100:13380–13383

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B (2005) Diverse taxa of cyanobacteria produce beta-N-methylamino-L-alanine, a neurotoxic amino acid. Proc Natl Acad Sci USA 102:5074–5078

Crews TE, Kurina LM, Vitousek PM (2001) Organic matter and nitrogen accumulation and nitrogen fixation during early ecosystem development in Hawaii. Biogeochemistry 52:259–279

Crittenden PD, Llimona X, Sancho LG (2007) Lichenized unicellular cyanobacteria fix nitrogen in the light. Can J Bot 85:1003–1006

Cusack DF, Silver W, McDowell WH (2009) Biological nitrogen fixation in two tropical forests: ecosystem-level patterns and effects of nitrogen fertilization. Ecosystems 12:1299–1315

Czeczuga B, Czeczuga-Semeniuk E, Semeniuk A (2006) Phycobiliprotein pigments in *Pseudocyphellaria* species and their role in complementary chromatic adaptation. J Hattori Bot Lab 100:625–633

Czeczuga B, Czeczuga-Semeniuk E, Semeniuk A (2010) Chromatic adaptation in lichen phyco- and photobionts. Biologia 65:587–594

Dahlman L, Nasholm T, Palmqvist K (2002) Growth, nitrogen uptake, and resource allocation in the two tripartite lichens *Nephroma arcticum* and *Peltigera aphthosa* during nitrogen stress. New Phytol 153:307–315

Dahlman L, Persson J, Palmqvist K, Nasholm T (2004) Organic and inorganic nitrogen uptake in lichens. Planta 219:459–467

DeLuca TH, Zackrisson O (2007) Enhanced soil fertility under *Juniperus communis* in arctic ecosystems. Plant Soil 294:147–155
DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419:917–920
DeLuca TH, Zackrisson O, Gentili F, Sellstedt A, Nilsson MC (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Oecologia 152:121–130
DeLuca TH, Zackrisson O, Gundale MJ, Nilsson M-C (2008) Ecosystem feedbacks and nitrogen fixation in boreal forests. Science 320:1181
den Herder M, Kytöviita MM, Niemela P (2003) Growth of reindeer lichens and effects of reindeer grazing on ground cover vegetation in a Scots pine forest and a subarctic heathland in Finnish Lapland. Ecography 26:3–12
DePriest PT (2004) Early molecular investigations of lichen-forming symbionts: 1986–2001. Annu Rev Microbiol 58:273–301
Diaz MC, Thacker RW, Rutzler K, Piantoni C (2007) Two new haplosclerid sponges from the Caribbean Panama with symbiotic filamentous cyanobacteria, and an overview of sponge-cyanobacteria associations. In: Custodio MR, Lobo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro, pp 31–39
Diaz E-M, Sacristán M, Legaz M-E, Vicente C (2009) Isolation and characterization of a cyanobacterium-binding protein and its cell wall receptor in the lichen *Peltigera canina*. Plant Signal Behav 4:598–603
Donia MS, Hathaway BJ, Sudek S, Haygood MG, Rosovitz MJ, Ravel J, Schmidt EW (2006) Natural combinatorial peptide libraries in cyanobacterial symbionts of marine ascidians. Nat Chem Biol 2:729–735
Donia MS, Fricke WF, Partensky F, Cox J, Elshahawi SI, White JR, Phillippy AM, Schatz MC, Piel J, Haygood MG, Ravel J, Schmidt EW (2011a) Complex microbiome underlying secondary and primary metabolism in the tunicate-*Prochloron* symbiosis. PNAS USA 108:1423–1432
Donia MS, Fricke WF, Ravel J, Schmidt EW (2011b) Variation in tropical reef symbiont metagenomes defined by secondary metabolism. PLoS One 6:e17897
Duckett JG, Burch J, Fletcher PW, Matcham HW, Read DJ, Russell AJ, Pressel S (2004) *In vitro* cultivation of bryophytes: a review of practicalities, problems, progress and promise. J Bryol 26:3–20
Duff RJ, Villarreal JC, Caagill DC, Renzaglia KS (2007) Progress and challenges toward developing a phylogeny and classification of the hornworts. Bryologist 110:214–243
Duggan PS, Gottardello P, Adams DG (2007) Molecular analysis of genes in *Nostoc punctiforme* involved in pilus biogenesis and plant infection. J Bacteriol 189:4547–4551
Ekman M, Tollback P, Klint J, Bergman B (2006) Protein expression profiles in an endosymbiotic cyanobacterium revealed by a proteomic approach. Mol Plant Microbe Interact 19:1251–1261
Ekman M, Tollback P, Bergman B (2008) Proteomic analysis of the cyanobacterium of the *Azolla* symbiosis: identity, adaptation, and NifH modification. J Exp Bot 59:1023–1034
Elifio SL, Da Silva M, Iacomini M, Gorin PAJ (2000) A lectin from the lichenized Basidiomycete *Dictyonema glabratum*. New Phytol 148:327–334
Ellis CJ, Coppins BJ (2006) Contrasting functional traits maintain lichen epiphyte diversity in response to climate and autogenic succession. J Biogeogr 33:1643–1656
Ellis CJ, Coppins BJ (2007) Changing climate and historic-woodland structure interact to control species diversity of the 'Lobarion' epiphyte community in Scotland. J Veg Sci 18:725–734
Ellis CJ, Yahr R, Coppins BJ (2009) Local extent of old-growth woodland modifies epiphyte response to climate change. J Biogeogr 36:302–313
Elvebakk A, Papaefthimiou D, Robertsen EH, Liaimer A (2008) Phylogenetic patterns among *Nostoc* cyanobionts within bi- and tripartite lichens of the genus *Pannaria*. J Phycol 44:1049–1059
Erwin PM, Thacker RW (2007) Incidence and identity of photosynthetic symbionts in Caribbean coral reef sponge assemblages. J Mar Biol Assoc UK 87:1683–1692
Erwin PM, Thacker RW (2008a) Phototrophic nutrition and symbiont diversity of two Caribbean sponge-cyanobacteria symbioses. Mar Ecol Prog Ser 362:139–147
Erwin PM, Thacker RW (2008b) Cryptic diversity of the symbiotic cyanobacterium *Synechococcus spongiarum* among sponge hosts. Mol Ecol 17:2937–2947
Erwin PM, Lopez-Legentil S, Gonzalez-Pech R, Turon X (2012) A specific mix of generalists: bacterial symbionts in Mediterranean *Ircinia* spp. FEMS Microbiol Ecol 79:619–637
Esterhuizen M, Downing TG (2008) beta-N-methylamino-L-alanine (BMAA) in novel South African cyanobacterial isolates. Ecotoxiocol Environ Saf 71:309–313
Falcón LI, Cipriano F, Chistoserdov AY, Carpenter EJ (2002) Diversity of diazotrophic unicellular cyanobacteria in the tropical North Atlantic Ocean. Appl Environ Microbiol 68:5760–5764
Fedrowitz K, Kaasalainen U, Rikkinen J (2011) Genotype variability of *Nostoc* symbionts associated with three epiphytic *Nephroma* species in a boreal forest landscape. Bryologist 114:220–230
Fine M, Loya Y (2002) Endolithic algae: an alternative source of photoassimilates during coral bleaching. Proc R Soc Lond Ser B 269:1205–1210
Flatt P, Gautschi J, Thacker R, Musafija-Girt M, Crews P, Gerwick W (2005) Identification of the cellular site of polychlorinated peptide biosynthesis in the marine sponge *Dysidea* (Lamellodysidea) *herbacea* and symbiotic cyanobacterium *Oscillatoria spongeliae* by CARD-FISH analysis. Mar Biol 147:761–774
Flores E, Herrero A (2005) Nitrogen assimilation and nitrogen control in cyanobacteria. Biochem Soc Trans 33:164–167
Flores E, Herrero A (2010) Compartmentalised function through cell differentiation in filamentous cyanobacteria. Nat Rev Microbiol 8:39–50
Fong P, Smith TB, Wartian MJ (2006) Epiphytic cyanobacteria maintain shifts to macroalgal dominance on coral reefs following ENSO disturbance. Ecology 87:1162–1168
Försterra F, Häussermann V (2008) Unusual symbiotic relationships between microendolithic phototrophic organisms and azooxanthellate cold-water corals from Chilean fjords. Mar Ecol Prog Ser 370:121–125
Foster RA, Zehr JP (2006) Characterization of diatom-cyanobacteria symbioses on the basis of *nifH*, *hetR* and 16S rRNA sequences. Environ Microbiol 8:1913–1925
Foster RA, Carpenter EJ, Bergman B (2006a) Unicellular cyanobionts in open ocean dinoflagellates, radiolarians, and tintinnids: ultrastructural characterization and immuno-localization of phycoerythrin and nitrogenase. J Phycol 42:453–463
Foster RA, Collier JL, Carpenter EJ (2006b) Reverse transcription PCR amplification of cyanobacterial symbiont 16S rRNA sequences from single non-photosynthetic eukaryotic marine planktonic host cells. J Phycol 42:243–250
Foster RA, Subramaniam A, Mahaffey C, Carpenter EJ, Capone DG, Zehr JP (2007) Influence of the Amazon River plume on distributions of free-living and symbiotic cyanobacteria in the western tropical North Atlantic Ocean. Limnol Oceanogr 52:517–532
Foster RA, Subramaniam A, Zehr JP (2009) Distribution and activity of diazotrophs in the Eastern Equatorial Atlantic. Environ Microbiol 11:741–750
Foster RA, Kuypers MMM, Vagner T, Paerl RW, Muzat N, Zehr JP (2011) Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses. ISME J 5:1484–1493. doi:10.1038/ismej.2011.26
Franche C, Lindstrom K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59
Frankovich TA, Armitage AR, Wachnicka AH, Gaiser EE, Fourqurean JW (2009) Nutrient effects on seagrass epiphyte community structure in Florida Bay. J Phycol 45:1010–1020

Freeman CJ, Thacker RW (2011) Complex interactions between marine sponges and their symbiotic microbial communities. Limnol Oceanog 56:1577–1586

Frias-Lopez J, Bonheyo GT, Jin Q, Fouke BW (2003) Cyanobacteria associated with coral black band disease in Caribbean and Indo-Pacific reefs. Appl Environ Microbiol 69:2409–2413

Friedrich AB, Fischer I, Proksch P, Hacker J, Hentschel U (2001) Temporal variation of the microbial community associated with the Mediterranean sponge *Aplysina aerophila*. FEMS Microbiol Ecol 38:105–113

Fuller DQ, Hickey LJ (2005) Systematics and leaf architecture of the Gunneraceae. Bot Rev 71:295–353

Furnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A (2008) Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. ISME J 2:561–570

Gaino E, Sciscioli M, Lepore E, Rebora M, Corriero G (2006) Association of the sponge *Tethya orphei* (Porifera, Demospongiae) with filamentous cyanobacteria. Invertebr Biol 125:281–287

Gantar M (2000a) Mechanical damage of roots provides enhanced colonization of the wheat endorhizosphere by the dinitrogen-fixing cyanobacterium *Nostoc* sp. strain 2S9B. Biol Fertil Soils 32:250–255

Gantar M (2000b) Co-cultivation of N_2-fixing cyanobacterium *Nostoc* sp strain 2S9B and wheat callus. Symbiosis 29:1–18

Garcia-Pichel F, Wade BD, Farmer JD (2002) Jet-suspended, calcite-ballasted cyanobacterial waterwarts in a desert spring. J Phycol 38:420–428

Gauslaa Y, Lie M, Solhaug K, Ohlson M (2006) Growth and ecophysiological acclimation of the foliose lichen *Lobaria pulmonaria* in forests with contrasting light climates. Oecologia 147:406–416

Gehringer MM, Pengelly JJL, Cuddy WS, Fieker C, Forster PI, Neilan BA (2010) Host selection of symbiotic cyanobacteria in 31 species of the Australian cycad genus: *Macrozamia* (Zamiaceae). Mol Plant Microbe Interact 23:811–822

Gentili F, Nilsson MC, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular diversity of feather moss associative N_2-fixing cyanobacteria. J Exp Bot 56:3121–3127

Golden JW, Yoon HS (2003) Heterocyst development in *Anabaena*. Curr Opin Microbiol 6:557–563

Gómez R, Erpenbeck D, Richelle-Maurer E, Van Dijk T, Woldringh CL, Van Soest RWM (2004) Affinities of *Aphanocapsa feldmanni*-like cyanobacteria from the marine sponge *Xestospongia muta* based on genetic and morphological analysis. In: Pansini M, Pronzato R, Bavestrello G, Manconi, R (eds) Sponge science in the new millenium, vol 68. Bollettino dei Musei e degli Instituti Biologici dell' Universita di Genova, pp 335–347

Gómez F, Furuya K, Takeda S (2005) Distribution of the cyanobacterium *Richelia intracellularis* as an epiphyte of the diatom *Chaetoceros compressus* in the western Pacific Ocean. J Plankton Res 27:323–330

Gorelova OA (2001) Surface ultrastructure of the heteromorphic cells of *Nostoc muscorum* CALU 304 in a mixed culture with the *Rauwolfia* callus tissue. Microbiology 70:285–294

Gorelova OA (2006) Communication of cyanobacteria with plant partners during association formation. Microbiology 75:465–469

Gorelova OA, Baulina OI (2009) Ultrastructure of cyanobacterium *Nostoc* sp f. *Blasia* cell forms in persisting populations. Microbiology 78:609–617

Gorelova OA, Kleimenov SY (2003) The accumulation and degradation dynamics of cyanophycin in cyanobacterial cells grown in symbiotic associations with plant tissues and cells. Microbiology 72:318–326

Gorelova OA, Korzhenevskaya TG (2002) Formation of giant and ultramicroscopic forms of *Nostoc muscorum* CALU 304 during cocultivation with *Rauwolfia* tissues. Microbiology 71:563–569

Goward T, Arsenault A (2000a) Cyanolichen distribution in young unmanaged forests: a dripzone effect? Bryologist 103:28–37

Goward T, Arsenault A (2000b) Cyanolichens and conifers: implications for global conservation. For Snow Landsc Res 75:303–318

Green TGA, Schlensog M, Sancho LG, Winkler JB, Broom FD, Schroeter B (2002) The photobiont determines the pattern of photosynthetic activity within a single lichen thallus containing cyanobacterial and green algal sectors (photosymbiodeme). Oecologia 130:191–198

Griffiths DJ (2006) Chlorophyll *b*-containing oxygenic photosynthetic prokaryotes: oxychlorobacteria (Prochlorophytes). Bot Rev 72:330–366

Grube M (2005) *Frigidopyrenia* – a new genus for a peculiar subarctic lichen, with notes on similar taxa. Phyton Ann Rei Bot 45:305–318

Grube M, Cardinale M, de Castro JV, Muller H, Berg G (2009) Species-specific structural and functional diversity of bacterial communities in lichen symbioses. ISME J 3:1105–1115

Gu WD, Kuusinen M, Konttinen T, Hanski I (2001) Spatial pattern in the occurrence of the lichen *Lobaria pulmonaria* in managed and virgin boreal forests. Ecography 24:139–150

Guevara R, Armesto JJ, Caru M (2002) Genetic diversity of *Nostoc* microsymbionts from *Gunnera tinctoria* revealed by PCR-STRR fingerprinting. Microb Ecol 44:127–136

Gundale MJ, Gustafsson H, Nilsson M-C (2009) The sensitivity of nitrogen fixation by a feathermoss-cyanobacteria association to litter and moisture variability in young and old boreal forests. Can J For Res 39:2542–2549

Gundale MJ, Deluca TH, Nordin A (2011) Bryophytes attenuate anthropogenic nitrogen inputs in boreal forests. Global Change Biol 17:2743–2753

Gusev MV, Baulina OI, Gorelova OA, Lobakova ES, Korzhenevshaya TG (2002) Artificial cyanobacterium-plant symbioses. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 253–312

Hamisi MI, Lyimo TJ, Muruke MHS, Bergman B (2009) Nitrogen fixation by epiphytic and epibenthic diazotrophs associated with seagrass meadows along the Tanzanian coast, Western Indian Ocean. Aquat Microb Ecol 57:33–42

Hardoim CCP, Costa R, Araujo FV, Hajdu E, Peixoto R, Lins U, Rosado AS, van Elsas JD (2009) Diversity of bacteria in the marine sponge *Aplysina fulva* in Brazilian coastal waters. Appl Environ Microbiol 75:3331–3343

Hashemloian BD, Azimi AA (2009) Alien and exotic *Azolla* in Northern Iran. Afr J Biotechnol 8:187–190

Hauck M, Spribille T (2002) The Mn/Ca and Mn/Mg ratios in bark, as possible causes for the occurrence of *Lobaria* lichens in the drip zone of *Populus* in western North America. Lichenologist 34:227–232

Hedenås H, Ericson L (2003) Response of epiphytic lichens on *Populus tremula* in a selective cutting experiment. Ecol Appl 13:1124–1134

Hedenås H, Ericson L (2004) Aspen lichens in agricultural and forest landscapes: the importance of habitat quality. Ecography 27:521–531

Hedenås H, Ericson L (2008) Species occurrences at stand level cannot be understood without considering the landscape context: cyanolichens on aspen in boreal Sweden. Biol Conserv 141:710–718

Hedenås H, Hedstrom P (2007) Conservation of epiphytic lichens: significance of remnant aspen (*Populus tremula*) trees in clear-cuts. Biol Conserv 135:388–395

Hedenås H, Blomberg P, Ericson L (2007) Significance of old aspen (*Populus tremula*) trees for the occurrence of lichen photobionts. Biol Conserv 135:380–387

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440

Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. FEMS Microbiol Ecol 55:167–177

Herrero A, Muro-Pastor AM, Valladares A, Flores E (2004) Cellular differentiation and the NtcA transcription factor in filamentous cyanobacteria. FEMS Microbiol Rev 28:469–487

Hill DJ (2009) Asymmetric co-evolution in the lichen symbiosis caused by a limited capacity for adaptation in the photobiont. Bot Rev 75:326–338

Hill M, Hill A, Lopez N, Harriott O (2006) Sponge-specific bacterial symbionts in the Caribbean sponge, *Chondrilla nucula* (Demospongiae, Chondrosida). Mar Biol 148:1221–1230

Hilmo O (2002) Growth and morphological response of old-forest lichens transplanted into a young and an old *Picea abies* forest. Ecography 25:329–335

Hilmo O, Ott S (2002) Juvenile development of the cyanolichen *Lobaria scrobiculata* and the green algal lichens *Platismatia glauca* and *Platismatia norvegica* in a boreal *Picea abies* forest. Plant Biol 4:273–280

Hindák F (2000) A contribution to the taxonomy of the nostocalean genus *Richelia* (Cyanophyta/Cyanobacteria). Biologia 55:1–6

Hirabayashi S, Kasai F, Watanabe MM, Hirose E (2006) Contents of ultraviolet-absorbing substances in two color morphs of the photosymbiotic ascidian *Didemnum molle*. Hydrobiologia 571:419–422

Hirose E (2000) Plant rake and algal pouch of the larvae in the tropical ascidian *Diplosoma similis*: an adaptation for vertical transmission of photosynthetic symbionts *Prochloron* sp. Zool Sci 17:233–240

Hirose E, Fukuda T (2006) Vertical transmission of photosymbionts in the colonial ascidian *Didemnum molle*: the larval tunic prevents symbionts from attaching to the anterior part of larvae. Zool Sci 23:669–674

Hirose E, Hirose M (2007) Morphological process of vertical transmission of photosymbionts in the colonial ascidian *Tridemnum miniatum* Kott, 1977. Mar Biol 150:359–367

Hirose E, Murakami A (2011) Microscopic anatomy and pigment characterization of coral-encrusting black sponge with cyanobacterial symbiont, *Terpios hoshinota*. Zool Sci 28:199–205

Hirose E, Nakabayashi S (2008) Algal symbionts in the larval tunic lamellae of the colonial ascidian *Lissoclinum timorense* (Ascidiacea, Didemnidae). Zool Sci 25:1205–1211

Hirose M, Ohtsuka K, Ishikura M, Maruyama T (2004) Ultraviolet absorption in ascidian tunic and ascidian-*Prochloron* symbiosis. J Mar Biol Assoc UK 84:789–794

Hirose E, Oka AT, Akahori M (2005) Sexual reproduction of the photosymbiotic ascidian *Diplosoma virens* in the Ryukyu Archipelago, Japan: vertical transmission, seasonal change, and possible impact of parasitic copepods. Mar Biol 146:677–682

Hirose E, Hirabayashi S, Hori K, Kasai F, Watanabe MM (2006a) UV protection in the photosymbiotic ascidian *Didemnum molle* inhabiting different depths. Zool Sci 23:57–63

Hirose E, Hirose M, Neilan BA (2006b) Localization of symbiotic cyanobacteria in the colonial ascidian *Tridemnum miniatum* (Didemnidae, Ascidiacea). Zool Sci 23:435–442

Hirose E, Adachi R, Kuze K (2006c) Sexual reproduction of the *Prochloron*-bearing ascidians, *Tridemnum cyclops* and *Lissoclinum bistratum*, in subtropical waters: seasonality and vertical transmission of photosymbionts. J Mar Biol Assoc UK 86:175–179

Hirose E, Oka AT, Hirose M (2009a) Two new species of photosymbiotic ascidians of the genus *Diplosoma* from the Ryukyu Archipelago, with partial sequences of the COI gene. Zool Sci 26:362–368

Hirose E, Uchida H, Murakami A (2009b) Ultrastructural and microspectrophotometric characterization of multiple species of cyanobacterial photosymbionts coexisting in the colonial ascidian *Tridemnum clinides* (Tunicata, Ascidiacea, Didemnidae). Eur J Phycol 44:365–375

Hobara S, McCalley C, Koba K, Giblin AE, Weiss MS, Gettel GM, Shaver GR (2006) Nitrogen fixation in surface soils and vegetation in an arctic tundra watershed: a key source of atmospheric nitrogen. Arct Antarct Alp Res 38:363–372

Holub SM, Lajtha K (2003) Mass loss and nitrogen dynamics during the decomposition of a N-^{15}-labeled N_2-fixing epiphytic lichen, *Lobaria oregana*. Can J Bot 81:698–705

Holub SM, Lajtha K (2004) The fate and retention of organic and inorganic ^{15}N-nitrogen in an old-growth forest soil in Western Oregon. Ecosystems 7:368–380

Houle D, Gauthier SB, Paquet S, Planas D, Warren A (2006) Identification of two genera of N_2-fixing cyanobacteria growing on three feather moss species in boreal forests of Quebec, Canada. Can J Bot 84:1025–1029

Hyvärinen M, Hardling R, Tuomi J (2002) Cyanobacterial lichen symbiosis: the fungal partner as an optimal harvester. Oikos 98:498–504

Janson S (2002) Cyanobacteria in symbiosis with diatoms. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 1–10

Janson S, Wouters J, Bergman B, Carpenter EJ (1999) Host specificity in the *Richelia*-diatom symbiosis revealed by *hetR* gene sequence analysis. Environ Microbiol 1:431–438

Johansson C, Bergman B (1992) Early events during the establishment of the *Gunnera*/*Nostoc* symbiosis. Planta 188:403–413

Jones AC, Gu LC, Sorrels CM, Sherman DH, Gerwick WH (2009) New tricks from ancient algae: natural products biosynthesis in marine cyanobacteria. Curr Opin Chem Biol 13:216–223

Jyothibabu R, Madhu NV, Maheswaran PA, Devi CRA, Balasubramanian T, Nair KKC, Achuthankutty CT (2006) Environmentally-related seasonal variation in symbiotic associations of heterotrophic dinoflagellates with cyanobacteria in the western Bay of Bengal. Symbiosis 42:51–58

Kaasalainen U, Jokela J, Fewer DP, Sivonen K, Rikkinen J (2009) Microcystin production in the tripartite cyanolichen *Peltigera leucophlebia*. Mol Plant Microbe Interact 22:695–702

Kappen L (2000) Some aspects of the great success of lichens in Antarctica. Antarct Sci 12:314–324

Karatygin IV, Snigirevskaya NS, Vikulin SV (2009) The most ancient terrestrial lichen *Winfrenatia reticulata*: a new find and new interpretation. Paleontol J 43:107–114

Karthikeyan N, Prasanna R, Nain L, Kaushik BD (2007) Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. Eur J Soil Biol 43:23–30

Karthikeyan N, Prasanna R, Sood A, Jaiswal P, Nayak S, Kaushik BD (2009) Physiological characterization and electron microscopic investigation of cyanobacteria associated with wheat rhizosphere. Folia Microbiol 54:43–51

Kennedy J, Marchesi JR, Dobson ADW (2007) Metagenomic approaches to exploit the biotechnological potential of the microbial consortia of marine sponges. Appl Microbiol Biotechnol 75:11–20

Kennedy J, Codling CE, Jones BV, Dobson ADW, Marchesi JR (2008) Diversity of microbes associated with the marine sponge, *Haliclona simulans*, isolated from Irish waters and identification of polyketide synthase genes from the sponge metagenome. Environ Microbiol 10:1888–1902

Khamar HJ, Breathwaite EK, Prasse CE, Fraley ER, Secor CR, Chibane FL, Elhai J, Chiu W-L (2010) Multiple roles of soluble sugars in the establishment of *Gunnera-Nostoc* endosymbiosis. Plant Physiol 154:1381–1389

Klaus JS, Janse I, Heikoop JM, Sanford RA, Fouke BW (2007) Coral microbial communities, zooxanthellae and mucus along gradients of seawater depth and coastal pollution. Environ Microbiol 9:1291–1305

Klint J, Ran L, Rasmussen U, Bergman B (2006) Identification of developmentally regulated proteins in cyanobacterial hormogonia using a proteomic approach. Symbiosis 41:87–95

Kluge M (2002) A fungus eats a cyanobacterium: the story of the *Geosiphon pyriformis* endocyanosis. Biol Environ Proc R Ir Acad 102B:11–14

Kluge M, Mollenhauer D, Wolf E, Shussler A (2002) The *Nostoc-Geosiphon* endocytobiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 19–30

Kneip C, Lockhart P, Voss C, Maier UG (2007) Nitrogen fixation in eukaryotes – new models for symbiosis. BMC Evol Biol 7. doi:10.1186/1471-2148-7-55

Kneip C, Voss C, Lockhart PJ, Maier UG (2008) The cyanobacterial endosymbiont of the unicellular algae *Rhopalodia gibba* shows reductive genome evolution. BMC Evol Biol 8. doi:10.1186/1471-2148-8-30

Knowles RD, Pastor J, Biesboer DD (2006) Increased soil nitrogen associated with dinitrogen-fixing, terricolous lichens of the genus *Peltigera* in northern Minnesota. Oikos 114:37–48

Knowlton N, Rohwer F (2003) Multispecies microbial mutualisms on coral reefs: the host as a habitat. Am Nat 162:S51–S62

Kojima A, Hirose E (2010) Transfer of prokaryotic algal symbionts from a tropical ascidian (*Lissoclinum punctatum*) colony to its larvae. Zool Sci 27:124–127

Kranner I, Beckett R, Hochman A, Nash TH (2008) Desiccation-tolerance in lichens: a review. Bryologist 111:576–593

Krings M, Hass H, Kerp H, Taylor TN, Agerer R, Dotzler N (2009) Endophytic cyanobacteria in a 400-million-yr-old land plant: a scenario for the origin of a symbiosis? Rev Paleobot Palynolog 153:62–69

Krüger T, Monch B, Oppenhauser S, Luckas B (2010) LC-MS/MS determination of the isomeric neurotoxins BMAA (beta-N-methylamino-L-alanine) and DAB (2,4-diaminobutyric acid) in cyanobacteria and seeds of *Cycas revoluta* and *Lathyrus latifolius*. Toxicon 55:547–557

Kucera P, Uher B, Komárek O (2005) Epiphytic cyanophytes *Xenococcus kerneri* and *Chamaesiphon minutus* on the freshwater red alga *Paralemanea catenata* (Rhodophyta). Biol Bratisl 60:1–3

Kühl M, Larkum AWD (2002) The microenvironment and photosynthetic performance of *Prochloron* sp. in symbiosis with didemnid ascidians. In: Seckbach J (ed) Cellular origin and life in extreme habitats. Kluwer Academic Publishers, Dordrecht, pp 273–290

Kühl M, Chen M, Ralph PJ, Schreiber U, Larkum AWD (2005) A niche for cyanobacteria containing chlorophyll *d*. Nature 433:820

Kurina LM, Vitousek PM (2001) Nitrogen fixation rates of *Stereocaulon vulcani* on young Hawaiian lava flows. Biogeochemistry 55:179–194

Kytöviita MM, Crittenden PD (2007) Growth and nitrogen relations in the mat-forming lichens S*tereocaulon paschale* and *Cladonia stellaris*. Ann Bot 100:1537–1545

Lagerstrom A, Nilsson MC, Zackrisson O, Wardle DA (2007) Ecosystem input of nitrogen through biological fixation in feather mosses during ecosystem retrogression. Funct Ecol 21:1027–1033

Lane AL, Moore BS (2011) A sea of biosynthesis: marine natural products meet the molecular age. Nat Prod Rep 28:411–428

Lange OL, Green TGA, Heber U (2001) Hydration-dependent photosynthetic production of lichens: what do laboratory studies tell us about field performance? J Exp Bot 52:2033–2042

Lange OL, Büdel B, Meyer A, Zellner H, Zotz G (2004) Lichen carbon gain under tropical conditions: water relations and CO_2 exchange of *Lobariaceae* species of a lower montane rainforest in Panama. Lichenologist 36:329–342

Larkum AWD, Kühl M (2005) Chlorophyll *d*: the puzzle resolved. Trends Plant Sci 10:355–357

Lechno-Yossef S, Nierzwicki-Bauer SA (2002) *Azolla-Anabaena* symbiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 153–178

Lee JJ (2006) Algal symbiosis in larger foraminifera. Symbiosis 42:63–75

Lee YK, Lee JH, Lee HK (2001) Microbial symbiosis in marine sponges. J Microbiol 39:254–264

Legaz M-E, Fontaniella B, Millanes A-M, Vicente C (2004) Secreted arginases from phylogenetically far-related species act as cross-recognition factors for two different algal cells. Eur J Cell Biol 83:435–446

Lehr H, Galun M, Ott S, Jahns HM, Fleminger G (2000) Cephalodia of the lichen *Peltigera aphthosa* (L.) Willd. Specific recognition of the compatible photobiont. Symbiosis 29:357–365

Lesser MP, Mazel CH, Gorbunov MY, Falkowski PG (2004) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science 305:997–1000

Lesser MP, Falcón LI, Rodriguez-Roman A, Enriquez S, Hoegh-Guldberg O, Iglesias-Prieto R (2007) Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*. Mar Ecol Prog Ser 346:143–152

Li AF, Tian ZJ, Li J, Yu RC, Banack SA, Wang ZY (2010) Detection of the neurotoxin BMAA within cyanobacteria isolated from freshwater in China. Toxicon 55:947–953

Li CQ, Liu WC, Zhu P, Yang JL, Cheng KD (2011) Phylogenetic diversity of bacteria associated with the marine sponge *Gelliodes carnosa* collected from the Hainan Island coastal waters of the South China Sea. Microb Ecol 62:800–812

Liaimer A, Bergman B (2004) Phytohormones in cyanobacteria: occurrence and perspectives. In: Tikhonovich I, Lugtenberg B, Provorov N (eds) Biology of plant-microbe interactions. International Society for Molecular Plant Microbe Interactions, St Paul, pp 394–397

Liaimer A, Matveyev A, Bergman B (2001) Isolation of host plant induced cDNAs from *Nostoc* sp strain PCC 9229 forming symbiosis with the angiosperm *Gunnera* spp. Symbiosis 31:293–307

Lindblad P, Costa J-L (2002) The cyanobacterial-cycad symbiosis. Biol Environ Proc R Ir Acad 102B:31–33

Lindlbad P, Bergman B, Hofsten AV, Hällblom L, Nylund JE (1985) The cyanobacterium-*Zamia* symbiosis: an ultrastructural study. New Phytol 101:707–716

Lindo Z, Whiteley JA (2011) Old trees contribute bio-available nitrogen through canopy bryophytes. Plant Soil 342:141–148

Lindquist N, Barber PH, Weisz JB (2005) Episymbiotic microbes as food and defence for marine isopods: unique symbioses in a hostile environment. Proc R Soc Lond B 272:1209–1216

Lindstrom EA, Johansen SW, Saloranta T (2004) Periphyton in running waters – long-term studies of natural variation. Hydrobiologia 521:63–86

Lobakova ES, Dol'nikova GA, Korzhenevshaya TG (2001a) Cyanobacterial-bacterial complexes in plant syncyanoses. Microbiology 70:111–116

Lobakova ES, Shchelmanova AG, Korzhenevskaya TG, Gusev MV (2001b) Infection of plants and plant tissue cultures with cyanobacteria-bacteria complexes. Microbiology 70:299–305

Lobakova ES, Orazova MK, Dobrovol'skaya TG (2003) Microbial complexes occurring on the apogeotropic roots and in the rhizosphere of cycad plants. Microbiology 72:628–633

Lohtander K, Oksanen I, Rikkinen J (2003) Genetic diversity of green algal and cyanobacterial photobionts in *Nephroma* (Peltigerales). Lichenologist 35:325–339

Lopez-Legentil S, Song B, Bosch M, Pawlik JR, Turon X (2011) Cyanobacterial diversity and a new *Acaryochloris*-like symbiont from Bahamian Sea-squirts. PLoS One 6:e23938

Lücking R (2008) Foliicolous lichenized fungi. Flora Neotrop Monogr 103:1–867

Lücking R, Lawrey JD, Sikaroodi M, Gillevet PM, Chaves JL, Sipman HJM, Bungartz F (2009) Do lichens domesticate photobionts like farmers domesticate crops? Evidence from a previously unrecognised lineage of filamentous cyanobacteria. Am J Bot 96:1409–1418

Lutzoni F, Pagel M, Reeb V (2001) Major fungal lineages are derived from lichen symbiotic ancestors. Nature 411:937–940

Mackenzie TDB, Campbell DA (2001) Evidence for wavelength-dependent light screening of cyanobionts and phycobionts in *Lobaria* during dehydration. Symbiosis 30:57–70

MacKenzie TDB, MacDonald TM, Dubois LA, Campbell DA (2001) Seasonal changes in temperature and light drive acclimation of photosynthetic physiology and macromolecular content in *Lobaria pulmonaria*. Planta 214:57–66

Mahaffey C, Michaels AF, Capone DG (2005) The conundrum of marine N_2 fixation. Am J Sci 305:546–595

Maldonado M (2007) Intergenerational transmission of symbiotic bacteria in oviparous and viviparous demosponges, with emphasis on intracytoplasmically-compartmented bacterial types. J Mar Biol Assoc UK 87:1701–1713

Marini L, Nascimbene J, Nimis PL (2011) Large-scale patterns of epiphytic lichen species richness: photobiont-dependent response to climate and forest structure. Sci Tot Environ 409:4381–4386

Markham JH (2009) Variation in moss-associated nitrogen fixation in boreal forest stands. Oecologia 161:353–359

Martinez-Garcia M, Koblizek M, Lopez-Legentil S, Anton J (2011) Epibiosis of oxygenic phototrophs containing chlorophylls a, b, c, and d on the colonial ascidian *Cystodytes dellechiajei*. Microb Ecol 61:13–19

Mattick JS (2002) Type IV pili and twitching motility. Annu Rev Microbiol 56:289–314

Matzek V, Vitousek P (2003) Nitrogen fixation in bryophytes, lichens and decaying wood along a soil-age gradient in Hawaiian montane rain forest. Biotropica 35:12–19

McCune B, Caldwell BA (2009) A single phosphorus treatment doubles growth of cyanobacterial lichen transplants. Ecology 90:567–570

McMurray SE, Blum JE, Leichter JJ, Pawlik JR (2011) Bleaching of the giant barrel sponge *Xestospongia muta* in the Florida Keys. Limnol Oceanog 56:2243–2250

Meeks JC (2003) Symbiotic interactions between *Nostoc punctiforme*, a multicellular cyanobacterium, and the hornwort *Anthoceros punctatus*. Symbiosis 35:55–71

Meeks JC (2009) Physiological adaptations in nitrogen-fixing *Nostoc*-plant symbiotic associations. In: Pawlowski K (ed) Prokaryotic symbionts in plants, vol 8, Microbiol monographs. Springer, Berlin, pp 181–205

Meeks JC, Elhai J (2002) Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. Microbiol Mol Biol Rev 66:94–121

Meeks JC, Elhai J, Thiel T, Potts M, Larimer F, Lamerdin J, Predki P, Atlas R (2001) An overview of the genome of *Nostoc punctiforme*, a multicellular, symbiotic cyanobacterium. Photosynth Res 70:85–106

Meeks JC, Campbell EL, Summers ML, Wong FC (2002) Cellular differentiation in the cyanobacterium *Nostoc punctiforme*. Arch Microbiol 178:395–403

Menge DNL, Hedin LO (2009) Nitrogen fixation in different biogeochemical niches along a 120,000-year chronosequence in New Zealand. Ecology 90:2190–2201

Metzgar JS, Schneider H, Pryer KM (2007) Phylogeny and divergence time estimates for the fern genus *Azolla* (Salvinaceae). Int J Plant Sci 168:1045–1053

Mohamed NM, Colman AS, Tal Y, Hill RT (2008) Diversity and expression of nitrogen fixation genes in bacterial symbionts of marine sponges. Environ Microbiol 10:2910–2921

Muggia L, Nelson P, Wheeler T, Yakovchenko LS, Tonsberg T, Spribille T (2011) Convergent evolution of a symbiotic duet: the case of the lichen genus *Polychidium* (Peltigerales, Ascomycota). Am J Bot 98:1647–1656

Münchhoff J, Hirose E, Maruyama T, Sunairi M, Burns BP, Neilan BA (2007) Host specificity and phylogeography of the prochlorophyte *Prochloron* sp., an obligate symbiont in didemnid ascidians. Environ Microbiol 9:890–899

Murakami A, Miyashita H, Iseki M, Adachi K, Mimuro M (2004) Chlorophyll d in an epiphytic cyanobacterium of red algae. Science 303:1633

Muro-Pastor MI, Reyes JC, Florencio FJ (2001) Cyanobacteria perceive nitrogen status by sensing intracellular 2-oxoglutarate levels. J Biol Chem 276:38320–38328

Muro-Pastor AM, Reyes JC, Florencio FJ (2005) Ammonium assimilation in cyanobacteria. Photosynth Res 83:135–150

Myers JL, Richardson LL (2009) Adaptaton of cyanobacteria to the sulfide-rich microenvironment of black band disease coral. FEMS Microbiol Ecol 67:242–251

Myllys L, Stenroos S, Thell A, Kuusinen M (2007) High cyanobiont selectivity of epiphytic lichens in old growth boreal forest of Finland. New Phytol 173:621–629

Nilsson MC, Wardle DA (2005) Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. Eur J Phycol 3:421–428

Nilsson M, Bergman B, Rasmussen U (2000) Cyanobacterial diversity in geographically related and distant host plants of the genus *Gunnera*. Arch Microbiol 173:97–102

Nilsson M, Bhattacharya J, Rai AN, Bergman B (2002) Colonization of roots of rice (*Oryza sativa*) by symbiotic *Nostoc* strains. New Phytol 156:517–525

Nilsson M, Rasmussen U, Bergman B (2005) Competition among symbiotic cyanobacterial *Nostoc* strains forming artificial associations with rice (*Oryza sativa*). FEMS Microbiol Lett 245:139–144

Nilsson M, Rasmussen U, Bergman B (2006) Cyanobacterial chemotaxis to extracts of host and nonhost plants. FEMS Microbiol Ecol 55:382–390

Nudleman E, Kaiser D (2004) Pulling together with type IV pili. J Mol Microbiol Biotechnol 7:52–62

O'Brien HE, Miadlikowska J, Lutzoni F (2005) Assessing host specialization in symbiotic cyanobacteria associated with four closely related species of the lichen fungus *Peltigera*. Eur J Phycol 40:363–378

Öckinger E, Niklasson M, Nilsson SG (2005) Is local distribution of the epiphytic lichen *Lobaria pulmonaria* limited by dispersal capacity or habitat quality? Biodivers Conserv 14:759–773

Ohkubo S, Miyashita H, Murakami A, Takeyama H, Tsuchiya T, Mimuro M (2006) Molecular detection of epiphytic *Acaryochloris* spp. on marine macroalgae. Appl Environ Microbiol 72:7912–7915

Oksanen I (2006) Ecological and biotechnological aspects of lichens. Appl Microbiol Biotechnol 73:723–734

Oksanen I, Lohtander K, Paulsrud P, Rikkinen J (2002) A molecular approach to cyanobacterial diversity in a rock-pool community involving gelatinous lichens and free-living *Nostoc* colonies. Ann Bot Fennici 39:93–99

Oksanen I, Jokela J, Fewer DP, Wahlsten M, Rikkinen J, Sivonen K (2004) Discovery of rare and highly toxic microcystins from lichen-associated cyanobacterium *Nostoc* sp strain IO-102-I. Appl Environ Microbiol 70:5756–5763

Olson ND, Ainsworth TD, Gates RD, Takabayashi M (2009) Diazotrophic bacteria associated with Hawaiian *Montipora* corals: diversity and abundance in correlation with symbiotic dinoflagellates. J Exp Mar Biol Ecol 371:140–146

Oren M, Steindler L, Ilan M (2005) Transmission, plasticity and the molecular identification of cyanobacterial symbionts in the Red Sea sponge *Diacarnus erythraenus*. Mar Biol 148:35–41

Osborne B, Sprent JI (2002) Ecology of the *Nostoc-Gunnera* symbiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 233–251

Ouyang YC, Dai SK, Xie LW, Kumar MSR, Sun W, Sun HM, Tang DL, Li X (2010) Isolation of high molecular weight DNA from marine sponge bacteria for BAC library construction. Mar Biotechnol 12:318–325

Pabby A, Prasanna BM, Nayak S, Singh AK (2003) Physiological characterization of the cultured and freshly isolated endosymbionts from different species of *Azolla*. Mar Biotechnol 41:73–79

Pabby A, Prasanna BM, Singh AK (2004a) Biological significance of *Azolla* and its utilization in agriculture. Proc Indian Natl Sci Acad B 3:299–333

Pabby A, Prasanna R, Singh PK (2004b) Morphological characterization of cultured and freshly separated cyanobionts (Nostocales, Cyanophyta) from *Azolla* species. Acta Bot Hung 46:211–223

Palmqvist K (2000) Carbon economy in lichens. New Phytol 148:11–36

Palmqvist K (2002) Cyanolichens: carbon metabolism. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 73–96

Palmqvist K, Dahlman L, Valladares F, Tehler A, Sancho LG, Mattsson JE (2002) CO_2 exchange and thallus nitrogen across 75 contrasting lichen associations from different climate zones. Oecologia 133:295–306

Papaefthimiou D, Hrouzek P, Mugnai MA, Lukesova A, Turicchia S, Rasmussen U, Ventura S (2008a) Differential patterns of evolution and distribution of the symbiotic behaviour in nostocacean cyanobacteria. Int J Syst Evol Microbiol 58:553–564

Papaefthimiou D, Van Hove C, Lejeune A, Rasmussen U, Wilmotte A (2008b) Diversity and host specificity of *Azolla* cyanobionts. J Phycol 44:60–70

Parsons R, Sunley RJ (2001) Nitrogen nutrition and the role of root-shoot nitrogen signalling particularly in symbiotic systems. J Exp Bot 52:435–443

Paulsrud P (2001) The *Nostoc* symbiont of lichens. Diversity, specificity and cellular modifications. Comprehensive summaries of Uppsal dissertations from the Faculty of Science and Technology 662. Acta Universitatis Upsalensis, Uppsala, pp 1–55

Paulsrud P, Lindblad P (2002) Fasciclin domain proteins are present in *Nostoc* symbionts of lichens. Appl Environ Microbiol 68:2036–2039

Paulsrud P, Rikkinen J, Lindblad P (2000) Spatial patterns of photobiont diversity in some *Nostoc*-containing lichens. New Phytol 146:291–299

Paulsrud P, Rikkinen J, Lindblad P (2001) Field investigations on cyanobacterial specificity in *Peltigera aphthosa*. New Phytol 152:117–123

Pereg-Gerk L, Sar N, Lipkin Y (2002) *In situ* nitrogen fixation associated with seagrasses in the Gulf of Elat (Red Sea). Aquat Ecol 36:387–394

Pereira AL, Carrapiço F (2007) Histochemistry of simple hairs from the foliar cavities of *Azolla filiculoides*. Plant Biosyst 141:323–328

Peterson EB, McCune B (2001) Diversity and succession of epiphytic macrolichen communities in low-elevation managed conifer forests in Western Oregon. J Veg Sci 12:511–524

Peterson EB, McCune B (2003) The importance of hotspots for lichen diversity in forests of western Oregon. Bryologist 106:246–256

Piel J (2006a) Combinatorial biosynthesis in symbiotic bacteria. Nat Chem Biol 2:661–662

Piel J (2006b) Bacterial symbionts: prospects for the sustainable production of invertebrate-derived pharmaceuticals. Curr Med Chem 13:39–50

Power M, Lowe R, Furey P, Welter J, Limm M, Finlay J, Bode C, Chang S, Goodrich M, Sculley J (2009) Algal mats and insect emergence in rivers under Mediterranean climates: towards photogrammetric surveillance. Freshw Biol 54:2101–2115

Prechtl J, Kneip C, Lockhart P, Wenderoth K, Maier UG (2004) Intracellular spheroid bodies of *Rhopalodia gibba* have nitrogen-fixing apparatus of cyanobacterial origin. Mol Biol Evol 21:1477–1481

Rai AN (2002) Cyanolichens: nitrogen metabolism. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 97–115

Rai AN, Bergman B (2002) Cyanolichens. Biol Environ Proc R Ir Acad 102B:19–22

Rai AN, Soderback E, Bergman B (2000) Cyanobacterium-plant symbioses. New Phytol 147:449–481

Ran L, Huang F, Ekman M, Klint J, Bergman B (2007) Proteomic analyses of the photoauto- and diazotrophically grown cyanobacterium *Nostoc* sp PCC 73102. Microbiol SGM 153:608–618

Ran LA, Larsson J, Vigil-Stenman T, Nylander JAA, Ininbergs K, Zheng WW, Lapidus A, Lowry S, Haselkorn R, Bergman B (2010) Genome erosion in a nitrogen-fixing vertically transmitted endosymbiotic multicellular cyanobacterium. Plos One 5(7). doi:10.1371/journal.pone.0011486

Rasmussen U, Johansson C (2002) Diversity and specificity in cyanobacterial symbioses. Biol Environ Proc R Ir Acad 102B:53–56

Rasmussen U, Nilsson M (2002) Cyanobacterial diversity and specificity in plant symbioses. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 313–328

Rasmussen U, Svenning MM (2001) Characterization by genotypic methods of symbiotic *Nostoc* strains isolated from five species of *Gunnera*. Arch Microbiol 176:204–210

Raven JA (2002a) The evolution of cyanobacterial symbioses. Biol Environ Proc R Ir Acad 102B:3–6

Raven JA (2002b) Evolution of cyanobacterial symbioses. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 329–346

Reid JD, Plunkett GM, Peters GA (2006) Phylogenetic relationships in the heterosporous fern genus *Azolla* (*Azollaceae*) based on DNA sequence data from three noncoding regions. Int J Plant Sci 167:529–538

Renzaglia KS, Duff RJ, Nickrent DL, Garbary D (2000) Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. Philos Trans R Soc Lond B 355:769–793

Renzaglia KS, Schuette S, Duff RJ, Ligrone R, Shaw AJ, Mishler BD, Duckett JG (2007) Bryophyte phylogeny: advancing the molecular and morphological frontiers. Bryologist 110:179–213

Richardson DHS, Cameron RP (2004) Cyanolichens: their response to pollution and possible management strategies for their conservation in northeastern North America. Northeast Nat 11:1–22

Ridley CP, Bergquist PR, Harper MK, Faulkner DJ, Hooper JNA, Haygood MG (2005a) Speciation and biosynthetic variation in four dictyoceratid sponges and their cyanobacterial symbiont, *Oscillatoria spongeliae*. Chem Biol 12:397–406

Ridley CP, Faulkner DJ, Haygood MG (2005b) Investigation of *Oscillatoria spongeliae*-dominated bacterial communities in four dictyoceratid sponges. Appl Environ Microbiol 71:7366–7375

Rikkinen J (2002) Cyanolichens: an evolutionary overview. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 31–72

Rikkinen J (2003) Ecological and evolutionary role of photobiont-mediated guilds in lichens. Symbiosis 34:99–110

Rikkinen J, Poinar GO (2002) Fossilised Anzia (Lecanorales, lichen-forming ascomycota) from European tertiary amber. Mycol Res 106:984–990

Rikkinen J, Poinar GOJ (2008) A new species of *Phyllopsora* (Lecanorales, lichen-forming mscomycota) from Dominican amber, with remarks on the fossil history of lichens. J Exp Bot 59:1007–1011

Rikkinen J, Virtanen V (2008) Genetic diversity in cyanobacterial symbionts of thalloid bryophytes. J Exp Bot 59:1013–1021

Rikkinen J, Oksanen I, Lohtander K (2002) Lichen guilds share related cyanobacterial symbionts. Science 297:357

Rohwer F, Breitbart M, Jara J, Azam F, Knowlton N (2001) Diversity of bacteria associated with the Caribbean coral *Montastraea franksi*. Coral Reefs 20:85–91

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10

Romagnoli T, Bavestrello G, Cucchiari EM, De Stefano M, Di Camillo CG, Pennesi C, Puce S, Totti C (2007) Microalgal communities epibiontic on the marine hydroid *Eudendrium racemosum* in the Ligurian Sea during an annual cycle. Mar Biol 151:537–552

Rosén J, Hellenäs KE (2008) Determination of the neurotoxin BMAA (beta-N-methylamino-L-alanine) in cycad seed and cyanobacteria by LC-MS/MS (liquid chromatography tandem mass spectrometry). Analyst 133:1785–1789

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362

Sacristan-Soriano O, Banaigs B, Becerro MA (2011) Relevant spatial scales of chemical variation in *Aplysina aerophoba*. Mar Drugs 9:2499–2513

Sacristán M, Millanes A-M, Legaz M-E, Vicente C (2006) A lichen lectin specifically binds to the alpha-1,4-polygalactoside moiety of urease located in the cell wall of homologous algae. Plant Signal Behav 1:23–27

Sanders WB (2001) Lichens: the interface between mycology and plant morphology. Bioscience 51:1025–1035

Sanders WB (2006) A feeling for the superorganism: expression of plant form in the lichen thallus. Bot J Linnean Soc 150:89–99

Sassaki GL, Gorin PAJ, Reis RA, Serrato RV, Elifio SL, Iacomini M (2005) Carbohydrate, glycolipid, and lipid components from the photobiont (*Scytonema* sp.) of the lichen, *Dictyomema glabratum*. Carbohydr Res 340:1808–1817

Schlensog M, Schroeter B, Green TGA (2000) Water dependent photosynthetic activity of lichens from New Zealand: differences in the green algal and the cyanobacterial thallus parts of photosymbiodemes. Bibl Lichenol 75:149–160

Schmidt EW, Nelson JT, Rasko DA, Sudek S, Eisen JA, Haygood MG, Ravel J (2005) Patellamide A and C biosynthesis by a microcin-like pathway in *Prochloron didemni*, the cyanobacterial symbiont of *Lissoclinum patella*. Proc Natl Acad Sci USA 102:7315–7320

Schmitt S, Angermeier H, Schiller R, Lindquist N, Hentschel U (2008) Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. Appl Environ Microbiol 74:7694–7708

Schnepf E, Schlegel I, Hepperle D (2002) *Petalomonas sphagnophila* (Euglenophyta) and its endocytobiotic cyanobacteria: a unique form of symbiosis. Phycologia 41:153–157

Schultz M (2007) On the identity of *Anema dodgei*, *Psorotichia segregata* and *Psorotichia squamulosa*, three misunderstood cyanolichens from the southwestern United States. Bryologist 110:286–294

Schultz M, Büdel B (2002) Key to the genera of the Lichinaceae. Lichenologist 34:39–62

Schultz M, Porembski S, Büdel B (2000) Diversity of rock-inhabiting cyanobacterial lichens: studies on granite inselbergs along the Orinoco and in Guyana. Plant Biol 2:482–495

Schüßler A (2002) Molecular phylogeny, taxonomy, and evolution of *Geosiphon pyriformis* and arbuscular mycorrhizal fungi. Plant Soil 244:75–83

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol Res 105:1413–1421

Schüßler A, Martin H, Cohen D, Fitz M, Wipf D (2006) Characterization of a carbohydrate transporter from symbiotic glomeromycotan fungi. Nature 444:933–936

Seifert GJ, Roberts K (2007) The biology of arabinogalactan proteins. Annu Rev Plant Biol 58:137–161

Selvin J, Ninawe AS, Kiran GS, Lipton AP (2010) Sponge-microbial interactions: ecological implications and bioprospecting avenues. Crit Rev Microbiol 36:82–90

Sergeeva E, Liaimer A, Bergman B (2002) Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. Planta 215:229–238

Shimada A, Yano N, Kanai S, Lewin RA, Maruyama T (2003) Molecular phylogenetic relationship between two symbiotic photo-oxygenic prokaryotes, *Prochloron* sp. and *Synechocystis trididemni*. Phycologia 42:193–197

Sillett SC, McCune B, Peck JE, Rambo TR, Ruchly A (2000) Dispersal limitations of epiphytic lichens result in species dependent on old-growth forests. Ecol Appl 10:789–799

Simmons TL, Coates RC, Clark BR, Engene N, Gonzalez D, Esquenazi E, Dorrestein PC, Gerwick WH (2008) Biosynthetic origin of natural products isolated from marine microorganism-invertebrate assemblages. Proc Natl Acad Sci USA 105:4587–4594

Snoeijs P, Murasi LW (2004) Symbiosis between diatoms and cyanobacterial colonies. Vie Et Milieu Life Environ 54:163–169

Solheim B, Zielke M (2002) Associations between cyanobacteria and mosses. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 137–152

Solheim B, Johanson U, Callaghan TV, Lee JA, Gwynn-Jones D, Björn LO (2002) The nitrogen fixation potential of arctic cryptogram species is influenced by enhanced UV-B radiation. Oecologia 133:90–93

Solheim B, Wiggen H, Roberg S, Spaink HP (2004) Associations between arctic cyanobacteria and mosses. Symbiosis 37:169–187

Solheim B, Zielke M, Bjerke JW, Rozema J (2006) Effects of enhanced UV-B radiation on nitrogen fixation in arctic ecosystems. Plant Ecol 182:109–118

Sood A, Prasanna R, Prasanna BM, Singh PK (2008a) Genetic diversity among and within cultured cyanobionts of diverse species of *Azolla*. Folia Microbiol 53:35–43

Sood A, Prasanna R, Singh PK (2008b) Fingerprinting of freshly separated and cultured cyanobionts from different *Azolla* species using morphological and molecular markers. Aquat Bot 88:142–147

Sood A, Singh PK, Kumar A, Singh R, Prasanna R (2011) Growth and biochemical characterization of associations between cyanobionts and wheat seedlings in co-culturing experiments. Biologia 66:104–110

Spáčil Z, Eriksson J, Jonasson S, Rasmussen U, Ilag LL, Bergman B (2010) Analytical protocol for identification of BMAA and DAB in biological samples. Analyst 135:127–132

Steindler L, Huchon D, Avni A, Ilan M (2005) 16S rRNA phylogeny of sponge-associated cyanobacteria. Appl Environ Microbiol 71:4127–4131

Steinke TD, Lubke RA, Ward CJ (2003) The distribution of algae epiphytic on pneumatophores of the mangrove, *Avicennia marina*, at different salinities in the Kosi System. S Afr J Bot 69:546–554

Stenroos S, Hognabba F, Myllys L, Hyvonen J, Thell A (2006) High selectivity in symbiotic associations of lichenized ascomycetes and cyanobacteria. Cladistics 22:230–238

Stewart KJ, Lamb EG, Coxson DS, Siciliano SD (2011) Bryophyte-cyanobacterial associations as a key factor in $N_{(2)}$-fixation across the Canadian Arctic. Plant Soil 344:335–346

Storeheier PV, Mathiesen S, Tyler N, Olsen M (2002) Nutritive value of terricolous lichens for reindeer in winter. Lichenologist 34:247–257

Summerfield TC, Eaton-Rye JJ (2006) *Pseudocyphellaria crocata*, *P. neglecta* and *P. perpetua* from the Northern and Southern Hemispheres are a phylogenetic species and share cyanobionts. New Phytol 170:597–607

Summerfield TC, Galloway DJ, Eaton-Rye JJ (2002) Species of cyanolichens from *Pseudocyphellaria* with indistinguishable ITS sequences have different photobionts. New Phytol 155:121–129

Sundberg B, Nasholm T, Palmqvist K (2001) The effect of nitrogen on growth and key thallus components in the two tripartite lichens, *Nephroma arcticum* and *Peltigera aphthosa*. Plant Cell Environ 24:517–527

Svenning MM, Eriksson T, Rasmussen U (2005) Phylogeny of symbiotic cyanobacteria within the genus *Nostoc* based on 16S rDNA sequence analyses. Arch Microbiol 183:19–26

Tang SL, Hong MJ, Liao MH, Jane WN, Chiang PW, Chen CB, Chen CA (2011) Bacteria associated with an encrusting sponge (*Terpios hoshinota*) and the corals partially covered by the sponge. Environ Microbiol 13:1179–1191

Tarangkoon W, Hansen G, Hansen PJ (2010) Spatial distribution of symbiont-bearing dinoflagellates in the Indian Ocean in relation to oceanographic regimes. Aquat Microb Ecol 58:197–213

Taylor TN, Krings M (2005) Fossil microorganisms and land plants: associations and interactions. Symbiosis 40:119–135

Taylor MW, Radax R, Steger D, Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol Mol Biol Rev 71:295–347

Thacker RW (2005) Impacts of shading on sponge-cyanobacteria symbioses: a comparison between host-specific and generalist associations. Integr Comp Biol 45:369–376

Thacker RW, Starnes S (2003) Host specificity of the symbiotic cyanobacterium *Oscillatoria spongeliae* in marine sponges, *Dysidea* spp. Mar Biol 142:643–648

Thacker RW, Diaz MC, Rutzler K, Erwin PM, Kimble SJA, Pierce MJ, Dillard SL (2007) Phylogenetic relationships among the filamentous cyanobacterial symbionts of Caribbean sponges and a comparison of photosynthetic production between sponges hosting filamentous and unicellular cyanobacteria. In: Porifera research: biodiversity, innovation and sustainability – 2007. Serie livros 28. Museu Nacional, Rio de Janeiro, pp 621–626

Thajuddin N, Muralitharan G, Sundaramoorthy M, Ramamoorthy R, Ramachandran S, Akbarsha MA, Gunasekaran M (2010) Morphological and genetic diversity of symbiotic cyanobacteria from cycads. J Basic Microbiol 50:254–265

Thiel V, Neulinger SC, Staufenberger T, Schmaljohann R, Imhoff JF (2007) Spatial distribution of sponge-associated bacteria in the Mediterranean sponge *Tethya aurantium*. FEMS Microbiol Ecol 59:47–63

Thurber RV, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, Dinsdale E, Kelly L, Rohwer F (2009) Metagenomic analysis of stressed coral holobionts. Environ Microbiol 11:2148–2163

Tripp HJ, Bench SR, Turk KA, Foster RA, Desany BA, Niazi F, Affourtit JP, Zehr JP (2010) Metabolic streamlining in an open-ocean nitrogen-fixing cyanobacterium. Nature 464:90–94

Tsavkelova EA, Cherdyntseva TA, Lobakova ES, Kolomeitseva GL, Netrusov AI (2001) Microbiota of the orchid rhizoplane. Microbiology 70:492–497

Tsavkelova EA, Lobakova ES, Kolomeitseva GL, Cherdyntseva TA, Netrusov AI (2003a) Localization of associative cyanobacteria on the roots of epiphytic orchids. Microbiology 72:86–91

Tsavkelova EA, Lobakova ES, Kolomeitseva GL, Cherdyntseva TA, Netrusov AI (2003b) Associative cyanobacteria isolated from the roots of epiphytic orchids. Microbiology 72:92–97

Uheda E, Maejima K (2009) Inhibition of nitrogen-fixing activity of the cyanobiont affects the localization of glutamine synthetase in hair cells of *Azolla*. J Plant Physiol 166:1705–1709

Uheda E, Silvester WB (2001) The role of papillae during the infection process in the *Gunnera-Nostoc* symbiosis. Plant Cell Physiol 42:780–783

Uheda E, Maejima K, Shiomi N (2004) Localization of glutamine synthetase isoforms in hair cells of *Azolla* leaves. Plant Cell Physiol 45:1087–1092

Uku J, Bjork M (2001) The distribution of epiphytic algae on three Kenyan seagrass species. S Afr J Bot 67:475–482

Uku J, Bjork M, Bergman B, Diez B (2007) Characterization and comparison of prokaryotic epiphytes associated with three East African seagrasses. J Phycol 43:768–779

Ungerer JL, Pratte BS, Thiel T (2008) Regulation of fructose transport and its effect on fructose toxicity in *Anabaena* spp. J Bacteriol 190:8115–8125

Untari LF, Suyono EA, Madyaratri P, Asri MN (2009) Identification of the symbiotic cyanobacteria (blue-green algae) in the root of the orchid *Spathoglottis plicata* BLUME. Phycologia 48:385

Usher KM (2008) The ecology and phylogeny of cyanobacterial symbionts in sponges. Mar Ecol Evol Perspect 29:178–192

Usher KM, Kuo J, Fromont J, Sutton DC (2001) Vertical transmission of cyanobacterial symbionts in the marine sponge *Chondrilla australiensis* (Demospongiae). Hydrobiologia 461:15–23

Usher KM, Fromont J, Sutton DC, Toze S (2004a) The biogeography and phylogeny of unicellular cyanobacterial symbionts in sponges from Australia and the Mediterranean. Microb Ecol 48:167–177

Usher KM, Toze S, Fromont J, Kuo J, Sutton DC (2004b) A new species of cyanobacterial symbiont from the marine sponge *Chondrilla nucula*. Symbiosis 36:183–192

Usher KM, Sutton DC, Toze S, Kuo J, Fromont J (2005) Inter-generational transmission of microbial symbionts in the marine sponge *Chondrilla australiensis* (Demospongiae). Mar Freshw Res 56:125–131

Usher KM, Kuo J, Fromont J, Toze S, Sutton DC (2006) Comparative morphology of five species of symbiotic and non-symbiotic coccoid cyanobacteria. Eur J Phycol 41:179–188

Usher KM, Bergman B, Raven JA (2007) Exploring cyanobacterial mutualisms. Annu Rev Ecol Evol Syst 38:255–273

Vaishampayan A, Sinha RP, Hader D-P, Dey T, Gupta AK, Bhan U, Rao AL (2001) Cyanobacterial biofertilizers in rice agriculture. Bot Rev 67:453–516

van Hove C, Lejeune A (2002a) The *Azolla-Anabaena* symbiosis. Biol Environ Proc R Ir Acad 102B:23–26

van Hove C, Lejeune A (2002b) Applied aspects of *Azolla-Anabaena* symbiosis. In: Rai AN, Bergman B, Rasmussen U (eds) Cyanobacteria in symbiosis. Kluwer Academic Publishers, Dordrecht, pp 179–193

Vazquez-Bermudez MF, Herrero A, Flores E (2002) 2-oxoglutarate increases the binding affinity of the NtcA (nitrogen control) transcription factor for the *Synechococcus glnA* promoter. FEBS Lett 512:71–74

Veerman J, Vasil'ev S, Paton GD, Ramanauskas J, Bruce D (2007) Photoprotection in the lichen *Parmelia sulcata*: the origins of desiccation-induced fluorescence quenching. Plant Physiol 145:997–1005

Venn AA, Loram JE, Douglas AE (2008) Photosynthetic symbioses in animals. J Exp Bot 59:1069–1080

Vessey JK, Pawlowski K, Bergman B (2005) Root-based N_2-fixing symbioses: legumes, actinorhizal plants, *Parasponia* sp and cycads. Plant Soil 274:51–78

Veys P, Lejeune A, Van Hove C (2000) The pore of the leaf cavity of *Azolla*: interspecific morphological differences and continuity between the cavity envelopes. Symbiosis 29:33–47

Veys P, Lejeune A, Van Hove C (2002) The pore of the leaf cavity of *Azolla* species: teat cell differentiation and cell wall projections. Protoplasma 219:31–42

Villarreal JC, Renzaglia KS (2006) Structure and development of *Nostoc* strands in *Leiosporoceros dussii* (Anthocerotophyta): a novel symbiosis in land plants. Am J Bot 93:693–705

Villarreal JC, Hassel de Menendez G, Allen NS (2007) *Nothoceros superbus* (Dendrocerotaceae), a new hornwort from Costa Rica. Bryologist 110:279–285

Vivas M, Sacristán M, Legaz M-E, Vicente C (2010) The cell recognition model in chlorolichens involving a fungal lectin binding to an algal ligand can be extended to cyanolichens. Plant Biol 12:615–621

Walsby AE (2007) Cyanobacterial heterocysts: terminal pores proposed as sites of gas exchange. Trends Microbiol 15:340–349

Wang CM, Ekman M, Bergman B (2004) Expression of cyanobacterial genes involved in heterocyst differentiation and dinitrogen fixation along a plant symbiosis development profile. Mol Plant Microbe Interact 17:436–443

Wanntorp L, Wanntorp HE (2003) The biogeography of *Gunnera* L.: vicariance and dispersal. J Biogeogr 30:979–987

Watts SD (2000) Signalling in the *Nostoc*-plant symbiosis. PhD thesis, University of Leeds, Leeds, UK, pp 185

Webster NS, Hill RT (2001) The culturable microbial community of the Great Barrier Reef sponge *Rhopaloeides odorabile* is dominated by an alpha-proteobacterium. Mar Biol 138:843–851

Wedin M, Wiklund E, Jorgensen PM, Ekman S (2009) Slippery when wet: phylogeny and character evolution in the gelatinous cyanobacterial lichens (Peltigerales, Ascomycetes). Mol Phylogenet Evol 53:862–871

Weiss M, Hobbie SE, Gettel GM (2005) Contrasting responses of nitrogen-fixation in arctic lichens to experimental and ambient nitrogen and phosphorus availability. Arct Antarct Alp Res 37:396–401

Werth S, Wagner HH, Gugerli F, Holderegger R, Csencsics D, Kalwij JM, Scheidegger C (2006) Quantifying dispersal and establishment limitation in a population of an epiphytic lichen. Ecology 87:2037–2046

White AE, Prahl FG, Letelier RM, Popp BN (2007) Summer surface waters in the Gulf of California: prime habitat for biological nitrogen fixation. Glob Biogeochem Cycles 21:GB2017. doi:10.1029/2006GB002840

Wikström N, Savolainen V, Chase MW (2001) Evolution of the angiosperms: calibrating the family tree. Proc R Soc Lond B 268:2211–2220

Williams CJ, Boyer JN, Jochem FJ (2009) Microbial activity and carbon, nitrogen, and phosphorus content in a subtropical seagrass (Florida Bay): evidence for limited bacterial use of seagrass production. Mar Biol 156:341–353

Wirtz N, Lumbsch HT, Green TGA, Turk R, Pintado A, Sancho L, Schroeter B (2003) Lichen fungi have low cyanobiont selectivity in maritime Antarctica. New Phytol 160:177–183

Wong FCY, Meeks JC (2001) The *hetF* gene product is essential to heterocyst differentiation and affects HetR function in the cyanobacterium *Nostoc punctiforme*. J Bacteriol 183:2654–2661

Wong FCY, Meeks JC (2002) Establishment of a functional symbiosis between the cyanobacterium *Nostoc punctiforme* and the bryophyte *Anthoceros punctatus* requires genes involved in nitrogen control and initiation of heterocyst differentiation. Microbiol SGM 148:315–323

Wouters J, Janson S, Bergman B (2000) The effect of exogenous carbohydrates on nitrogen fixation and *hetR* expression in *Nostoc* PCC 9229 forming symbiosis with *Gunnera*. Symbiosis 28:63–76

Wouters J, Bergman B, Janson S (2003) Cloning and expression of a putative cyclodextrin glucosyltransferase from the symbiotically competent cyanobacterium *Nostoc* sp PCC 9229. FEMS Microbiol Lett 219:181–185

Wouters J, Raven JA, Minnhagen S, Janson S (2009) The luggage hypothesis: comparisons of two phototrophic hosts with nitrogen-fixing cyanobacteria and implications for analogous life strategies for kleptoplastids/secondary symbiosis in dinoflagellates. Symbiosis 49:61–70

Wulff J (2006) Ecological interactions of marine sponges. Can J Zool Rev Can Zool 84:146–166

Yellowlees D, Rees TAV, Leggat W (2008) Metabolic interactions between algal symbionts and invertebrate hosts. Plant Cell Environ 31:679–694

Yokobori S, Kurabayashi A, Neilan BA, Maruyama T, Hirose E (2006) Multiple origins of the ascidian-*Prochloron* symbiosis: molecular phylogeny of photosymbiotic and non-symbiotic colonial ascidians inferred from 18S rDNA sequences. Mol Phylogenet Evol 40:8–19

Yuan XL, Xiao SH, Taylor TN (2005) Lichen-like symbiosis 600 million years ago. Science 308:1017–1020

Zackrisson O, DeLuca TH, Nilsson MC, Sellstedt A, Berglund LM (2004) Nitrogen fixation increases with successional age in boreal forests. Ecology 85:3327–3334

Zackrisson O, DeLuca TH, Gentili F, Sellstedt A, Jaderlund A (2009) Nitrogen fixation in mixed *Hylocomium splendens* moss communities. Oecologia 160:309–319

Zehr JP, Carpenter EJ, Villareal TA (2000) New perspectives on nitrogen-fixing microorganisms in tropical and subtropical oceans. Trends Microbiol 8:68–73

Zehr JP, Bench SR, Carter BJ, Hewson I, Niazi F, Shi T, Tripp JH, Affourtit JP (2008) Globally distributed uncultured oceanic N_2-fixing cyanobacteria lack oxygenic photosystem II. Science 322:1110–1112

Zhang C-C, Laurent S, Sakr S, Peng L, Bedu S (2006) Heterocyst differentiation and pattern formation in cyanobacteria: a chorus of signals. Mol Microbiol 59:367–375

Zheng WW, Song TY, Bao XD, Bergman B, Rasmussen U (2002) High cyanobacterial diversity in coralloid roots of cycads revealed by PCR fingerprinting. FEMS Microbiol Ecol 40:215–222

Zheng WW, Bergman B, Chen B, Zheng SP, Xiang G, Rasmussen U (2009a) Cellular responses in the cyanobacterial symbiont during its vertical transfer between plant generations in the *Azolla microphylla* symbiosis. New Phytol 181:53–61

Zheng WW, Ran L, Bergman B (2009b) Structural characteristics of the cyanobacterium-*Azolla* symbiosis. In: Pawlowski K (ed) Prokaryotic symbionts in plants, vol 8, Microbiol monographs. Springer, Berlin/Heidelberg, pp 235–263

Zhu P, Quanzi L, Quangyi W (2008) Unique microbial signatures of the alien Hawaiian marine sponge *Suberites zeteki*. Microb Ecol 55:406–414

Zielke M, Ekker AS, Olsen RA, Spjelkavik S, Solheim B (2002) The influence of abiotic factors on biological nitrogen fixation in different types of vegetation in the High Arctic, Svalbard. Arct Antarct Alp Res 34:293–299

Zielke M, Solheim B, Spjelkavik S, Olsen RA (2005) Nitrogen fixation in the high arctic: role of vegetation and environmental conditions. Arct Antarct Alp Res 37:372–378

Part V

Applied Aspects

Cyanotoxins

24

James S. Metcalf and Geoffrey A. Codd

Contents

J.S. Metcalf (✉)
Institute for Ethnomedicine, Box 3464, Jackson WY 83001, USA
e-mail: james@ethnomedicine.org

G.A. Codd
Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee DD1 4HN, UK
e-mail: g.a.codd@dundee.ac.uk

Summary

Cyanobacteria produce a wide range of bioactive compounds in marine, transient, freshwater and terrestrial ecosystems. Some of these compounds show very high toxicity (μg kg^{-1} body weight) in mammalian systems via a variety of molecular and pathogenic mechanisms. The health significance of these products, which include genotoxic-, tumour-promoting-, hepato- and neurotoxic agents, is confirmed by the continuing occurrence of associated human and animal toxicoses. Here, we review the range of toxins produced by cyanobacteria (cyanotoxins), their production, analysis, multiple fates and possible environmental significance in aquatic ecosystems. Gaps in knowledge are identified and progress in the risk management of cyanotoxins is also considered.

24.1 Introduction

Among the reasons why cyanobacteria continue to attract a great deal of scientific and public attention is the wide range of low molecular weight compounds (cyanotoxins) they can produce with adverse effects on humans, animals, plants and eukaryotic microbes. Since the first edition of this volume (Whitton and Potts 2000), there has been a substantial growth in research and publications on cyanotoxins. Reviews and other publications providing links to the more recent literature include Codd et al. (2005a, b), Zurawell et al. (2005),

B.A. Whitton (ed.), *Ecology of Cyanobacteria II: Their Diversity in Space and Time*,
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van Appeldoorn et al. (2007), Smith et al. (2008), Funari and Testai (2008), Hudnell (2008), Bownik (2010) and Kehr et al. (2011). The primary research advances in this period have been followed by increases in outreach activities, such as raising public awareness and risk management. These include international situation assessment and measures to protect public and environmental health and wellbeing (e.g. Falconer 2004; Metcalf and Codd 2004; Codd et al. 2005c; Carmichael 2008).

Traditionally, most research on cyanotoxins has focussed on two major groups of secondary metabolites, hepatotoxins and neurotoxins. Much of our knowledge about cyanotoxins has centred on their occurrence and actions in aquatic environments. This results from the fact that most of the toxins isolated and characterised, and of the associated human and animal health incidents, have originated from or occurred in aquatic ecosystems. However, recent studies indicate the need for a wider understanding of cyanotoxins, including exposure media and exposure routes. This review aims to provide a broad perspective on the principal cyanotoxin families known to present hazards to human health or which contribute to the adverse economic impacts of mass growths of cyanobacteria in natural and controlled environments. A vast array of other cyanobacterial secondary metabolites is emerging in screening and biodiversity research programmes. Many such products, including enzyme inhibitors, info- and allelochemicals, have adverse effects on other biological systems. If they impair biological function under environmentally-relevant conditions, then such products may be considered as toxins and links are provided to this growing archive of cyanobacterial bioactive metabolites.

24.2 What Are Toxic Cyanobacteria and What Is a Cyanotoxin?

Environmental samples of cyanobacteria identified in field monitoring and laboratory research strains used are often referred to as either "toxic" or "non-toxic" (e.g. Davis et al. 2009; Liu et al. 2009). In an ecological context, these terms might refer to the causation, or otherwise, of adverse effects on biota by intact cyanobacterial cells, cell lysates, or specific cyanotoxins, under environmentally-relevant conditions of exposure and dosage. The terms toxic and non-toxic are also used in decision-making as to whether a waterbody is acceptable for particular usage e.g. recreation, fisheries, or abstraction for drinking water treatment. With the move from the bioassay of crude cyanobacterial extracts towards the analysis of specific cyanotoxins, these broad terms are generally used to refer to the presence and concentration of a defined cyanotoxin or, more commonly, cyanotoxin family.

The most common criterion used in defining the toxicity of the analyte is usually its effect(s) on vertebrate-, especially mammalian-, test systems. However, cyanobacteria are now recognised to produce an array of compounds which are not toxic to mammals at environmentally-relevant concentrations, but which can have deleterious effects on other biota e.g. aquatic invertebrates. For example, the depsipeptide microviridin J of *Microcystis aeruginosa* and the cyclic peptide oscillapeptin J of *Planktothrix rubescens* are toxic to *Daphnia* sp. and *Eudiaptomus* sp., respectively (Rohrlack et al. 2003, 2004; Blom et al. 2006). Whilst these products are not likely to be "toxins" in relation to mammals, they are certainly so in relation to these invertebrates.

If they are defined, the terms toxic and non-toxic can be of value in decision-making as to whether a waterbody is acceptable for a particular purpose such as recreation, fisheries or abstraction for drinking water treatment. The concepts can also be a useful shorthand in research, if defined e.g. as "microcystin-producing", versus "non-microcystin-producing" (e.g. Chen et al. 2009). However, the known cyanotoxins of high toxicity to vertebrates such as microcystins can be produced either alongside, or in the absence of, the bioactive metabolites which are toxic to other biota. Thus, it is necessary to consider the environmental context and potential target organism(s) when applying the terms toxic and non-toxic to cyanobacterial strains, natural populations and extracted metabolites.

It follows that there is no single brief definition of a cyanotoxin other than it is a compound produced by cyanobacteria which has detrimental biological activity in a given environmental or test system. Some cyanotoxins (e.g. saxitoxins and lipopolysaccharide endotoxins) are not exclusive products of cyanobacteria, whereas others, such as the much studied microcystins and anatoxins, are not known elsewhere.

24.3 Toxins Produced by Cyanobacteria

24.3.1 Overview

The known and most potent cyanotoxins are largely low molecular weight alkaloids and cyclic peptides. Their names have mostly been derived from the original producer organism and at present the functions of the cyanotoxins in cyanobacteria are not known. Cyanotoxins can be found within the producer-cells or released into the external environment, such as water or spent growth media. They have been found in all environments tested and are considered to be an ancient, widespread phenomenon. A number of these compounds show high toxicity in mammalian systems and as such, some have been defined as Scheduled Chemical Weapons according to organisations such as The Australia Group. Increasingly, national and international legislation around the World is including cyanotoxins as potential bioterrorism agents which are, or may become, subject to regulation and control (Metcalf et al. 2006a; Metcalf and Codd 2009).

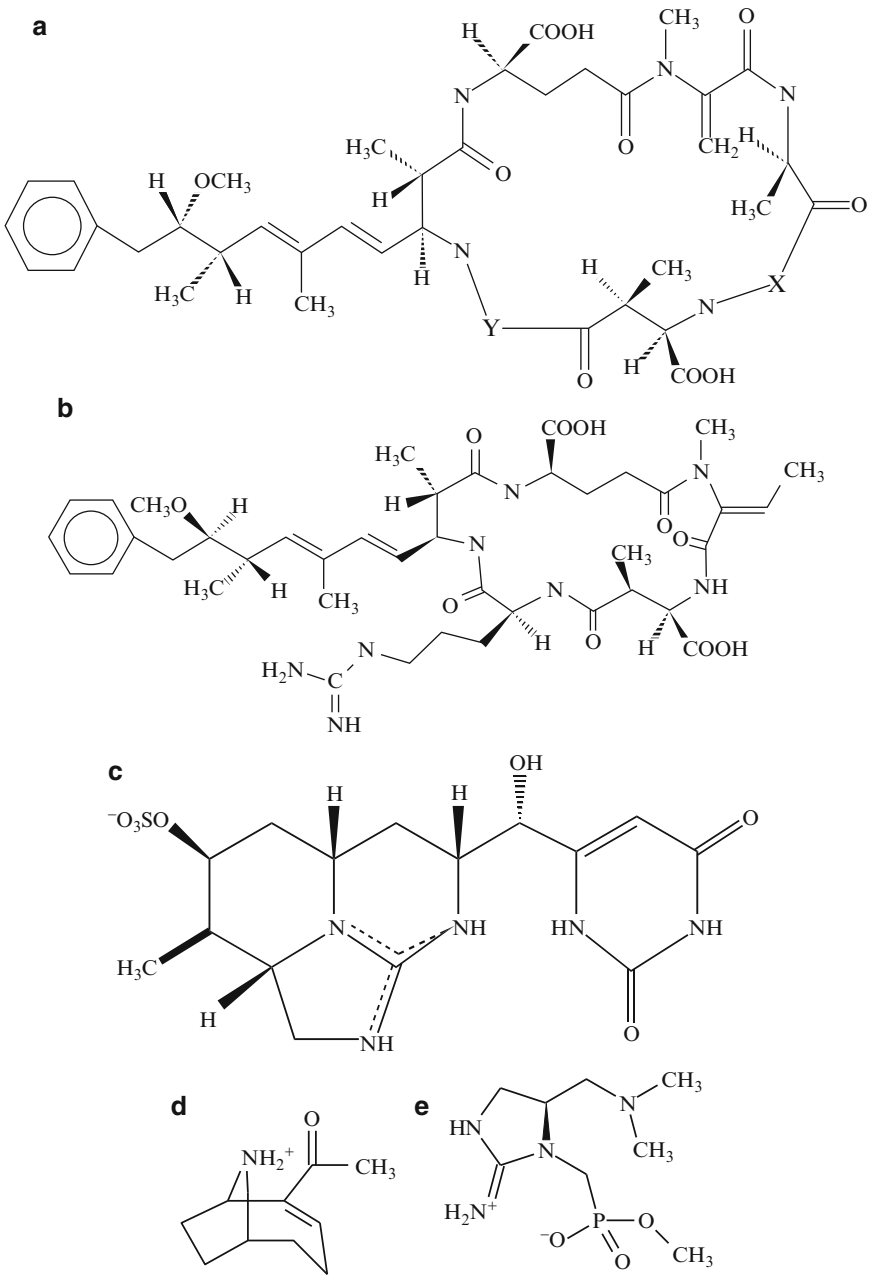

Fig. 24.1 Major classes of cyanotoxins. (**a**) microcystins; (**b**) nodularins; (**c**) cylindrospermopsins; (**d**) anatoxin-a; (**e**) anatoxin-a(S)

24.3.2 Microcystins and Nodularins

These cyclic peptides comprise two groups of molecules, the cyclic heptapeptide microcystins and the cyclic pentapeptide nodularins (Fig. 24.1). The microcystins have the general structure cyclo(-D-Ala-L-X-D-erythro-β-methylAsp(iso-linkage)-L-Y-Adda-D-Glu(iso-linkage)-*N*-methyldehydro-Ala) where Adda is the novel β-amino acid, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4 (*E*), 6(*E*)-dienoic acid and X and Y are sites of amino acid substitutions at positions 2 and 4 of the peptide ring. For example, microcystin-LR possesses L-leucine and L-arginine at the variable amino acid positions and this is the basis for the nomenclature of microcystin variants (Carmichael et al. 1988). Nodularins, which are two amino acids smaller than microcystins, have the general structure cyclo(-D-erythro-β-methylAsp(iso-linkage)-L-Y-Adda-D-Glu(iso-linkage)-2-methylamino-2(Z)-dehydrobutyric acid). The nodularins generally show less structural variability than the microcystins and L-Arg is often the amino acid at position Y in nodularin-R (Sivonen and Jones 1999).

In mammals, microcystins and nodularins in acute doses can cause death by hypovolaemic shock. However, although their uptake by a wide range of animal, plant and microbial cells and tissues occurs, toxin uptake occurs most readily,

both *in vitro* and *in vivo,* into liver cells via an organic anion transporting polypeptide (OATP) system (Runnegar et al. 1991). A pronounced sign of acute intoxication by microcystins and nodularins in mammals is the destruction of liver macrostructure by the deformation of the cytoskeleton in the hepatocytes, due to the hyperphosphorylation of cytoskeletal proteins (Humpage 2008). As a result, the majority of the circulating blood remains within the liver such that there is insufficient for the remainder of the body's organs to remain viable.

The traditional description of the microcystins and nodularins as hepatotoxins is no longer sufficient to describe their actions in animals. Microcystins can accumulate in multiple organs and tissues in mammals and fish: heart, liver, gonad, lung, brain and kidney with consequent physiological, tissue and cell damage (Wang et al. 2008; Zhao et al. 2009). In addition to OATP-mediated microcystin uptake into liver and kidney cells, it appears that an analogous OATP system is involved in the uptake of several microcystin variants, with increasing toxicity -LF > -LW, > -LR, in mouse primary whole brain cells (Feurstein et al. 2009). Whether microcystins (and nodularins) can thereby cross the blood-brain-barrier and blood-cerebrospinal fluid-barrier and thus account for neurological symptoms of toxicity in mammals requires investigation. Further signs of microcystin intoxication include damage to mitochondrial membranes and electron transport function (Humpage 2008; Zhao et al. 2008) and oxidative stress (Wiegand and Pflugmacher 2005; Zhao et al. 2009). Microcystins and nodularins also act as potent liver and colon tumour promoters in animal models when initiated by the carcinogen *N*-methyl-*N*-nitrosourea. These findings are consistent with the increase in proto-oncogene expression in rats in response to intravenous administration of partially-purified microcystins (Li et al. 2009). Whilst microcystin alone does not appear to be a carcinogen, weak carcinogenicity is caused by nodularin (van Appeldoorn et al. 2007; Humpage 2008).

At the molecular level, microcystins and nodularins are potent inhibitors of eukaryotic protein phosphatases including PP1, PP2A (Mackintosh et al. 1990) and phosphoprotein phosphatases PPP4 and PPP5 (Hastie et al. 2005). The full toxicological significance of this inhibition by the cyanotoxins is still not apparent since these phosphatases are highly conserved throughout eukaryotes and are widely involved in the regulation of cell processes, at genetic, developmental, metabolic and physiological levels, including tumour suppression (Gee and Mansuy 2005; Moorhead et al. 2007).

Microcystin production appears to be widespread, though sporadic, throughout cyanobacterial taxa, with examples from fresh, transient and marine waters, and terrestrial environments. Microcystin-producers in the latter include free-living (Metcalf et al. 2012) and symbiotic cyanobacteria (Table 24.1). Nodularin production, by contrast, currently appears to be restricted to *Nodularia spumigena*, with the possibility of a similar pentapeptide in the marine sponge *Theonella*, originating from a cyanobacterial symbiont (Table 24.1).

Table 24.1 Cyanobacteria known to produce the major classes of cyanotoxins (Adapted from Sivonen and Jones 1999; Dow and Swoboda 2000; Codd et al. 2005b)

Toxin	Published producers
Microcystins	**Chroococcales**: *Microcystis* spp., *M. aeruginosa*, *M. viridis*
	Oscillatoriales: *Oscillatoria* (*Planktothrix*) *agardhii*, *Plectonema boryanum*, *Phormidium corium,*[a] *Phormidium splendidum,*[b] *Arthrospira fusiformis*[c]
	Nostocales: *Anabaena* sp., *Anabaena flos-aquae*, *A. subcylindrica,*[a] *A. variabilis,*[a] *Nostoc* sp., *Nostoc spongiaeforme,*[a] *Anabaenopsis* sp., *Gloeotrichia echinulata,*[d] *Rivularia biasolettiana,*[b] *R. haematites,*[b] *Tolypothrix distorta*[b]
	Stigonematales: *Hapalosiphon* sp.
Nodularins	**Nostocales**: *Nodularia spumigena*
Anatoxin-a and homoanatoxin-a	**Oscillatoriales**: *Arthrospira fusiformis*, *Phormidium formosum,*[e] *Phormidium* sp., *Oscillatoria* sp.
	Nostocales: *Anabaena* spp., *Aphanizomenon* sp., *Anabaena flos-aquae*, *Anabaena planktonica*, *Cylindrospermum* sp., *Raphidiopsis mediterranea*,
Anatoxin-a(*S*)	**Nostocales**: *Anabaena flos-aquae*, *Anabaena lemmermannii*
Saxitoxins	**Oscillatoriales**: *Lyngbya wollei*, *Planktothrix* sp.
	Nostocales: *Aphanizomenon flos-aquae*, *Anabaena circinalis*, *Cylindrospermopsis raciborskii*
Cylindrospermopsins	**Nostocales**: *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, *Anabaena* sp., *Anabaena lapponica,*[f,g] *Raphidiopsis curvata*
	Stigonematales: *Umezakia natans*

[a]Mohamed et al. (2006), [b]Aboal et al. (2005), [c]Ballot et al. (2004), [d]Carey et al. (2007), [e]Skulberg et al. (1992), [f]Spoof et al. (2006), [g]Kokociński et al. (2009)

24.3.3 Cylindrospermopsin

After an outbreak of hepatic enteritis and the identification of *Cylindrospermopsis raciborskii* in the water source as a potential cause, this cyanobacterium was found to be toxic to mice and a toxic guanidine alkaloid, cylindrospermopsin was identified (Fig. 24.1; Hawkins et al. 1985). This cyanotoxin has since been identified in members of several cyanobacterial genera from aquatic environments (Table 24.1). Although cylindrospermopsins were considered to be a product of cyanobacterial blooms in tropical/subtropical environments,

the toxin is now being reported in temperate and boreal latitudes. This widening awareness may be partly due to increasing application of competent analytical methods and the identification of cylindropsermopsin in members of at least five planktonic genera (Table 24.1). At present, three structural variants of cylindrospermopsin have been identified: cylindrospermopsin, 7-epicylindrospermopsin and deoxycylindrospermopsin. Cylindrospermopsin and 7-epicylindrospermopsin have similar toxicities in mammalian systems and deoxycylindrospermopsin is considered non-toxic (Norris et al. 1999). Toxicity assessment of breakdown products of cylindrospermopsin has indicated that the uracil moiety of the molecule is necessary for toxicity (Banker et al. 2001). The pathology of mice administered with cylindrospermopsin indicated that this cyanotoxin causes multiple organ-, and tissue damage (Falconer and Humpage 2005). Cylindrospermopsin inhibits protein synthesis in animals and plants (Terao et al. 1994; Metcalf et al. 2004). Other concerns with cylindrospermopsin are that it is genotoxic *in vitro* (DNA strand breakage and whole chromosome-loss) and that it may be a carcinogen (Humpage and Falconer 2003; Humpage 2008). Evidence for carcinogenicity (Humpage 2008), and cytoskeletal and nuclear changes in Chinese hamster ovary cells (Gácsi et al. 2009), confirm the need to further characterise and quantify cylindropsermopsin toxicity.

24.3.4 Anatoxin-a and Homoanatoxin-a

A number of low molecular weight toxic alkaloids have been isolated from cyanobacteria, including the nicotinic agonist anatoxin-a (Fig. 24.1). The first toxin to be structurally and pharmacologically characterised from a cyanobacterium (Carmichael et al. 1975, 1979), this neurotoxin (molecular weight 165 Da) acts rapidly in mammalian and avian systems, with a toxic dose being able to kill mice in under 20 min. Anatoxin-a acts by competing with acetylcholine at neuromuscular junctions. Consequently, when anatoxin-a binds to acetylcholine receptors, acetylcholine esterase is unable to remove the cyanotoxin from neurons. As a result, the neurons continue to propagate neural pulses, leading to nerve depolarisation. Without therapeutic intervention, and in sufficient doses, the paralysis caused by anatoxin-a will lead to death by respiratory arrest. Such paralysis has been observed for example in birds, where contraction of muscles at the base of the neck results in opisthotonous: the forced positioning of the bird's neck and head along its back. Anatoxin-a has been identified in planktonic species/strains of *Anabaena*, *Arthrospira*, *Aphanizomenon*, *Planktothrix* and *Raphidiopsis*, and in mats of benthic *Oscillatoria* (*Phormidium*). A methylated variant of anatoxin-a, namely homoanatoxin-a, is of similar toxicity to anatoxin-a (Skulberg et al. 1992). 4-Hydroanatoxin-a and the breakdown products dihydroanatoxin-a and epoxyanatoxin-a also have been described (James et al. 1998; Furey et al. 2003). Although not found as frequently as microcystins, anatoxin-a and homoanatoxin-a continue to be identified as proximal or contributory causes of rapid toxicoses of domestic and wild animals (Sect. 24.4.1).

24.3.5 Anatoxin-a(S)

The phosphorylated cyclic *N*-hydroxyguanine anatoxin-a(S) (Fig. 24.1) is a potent acetylcholinesterase inhibitor. In common with synthetic organophosphorus pesticides, anatoxin-a(S) causes hypersalivation (hence the suffix "S") and lachrymation in mammals. Anatoxin-a(S) production has so far only been found in strains of *Anabaena flos-aquae* and *Anabaena lemmermannii* (Mahmood and Carmichael 1986, 1987; Matsunaga et al. 1989). Anatoxin-a(S) has been found to be responsible for wildlife poisonings. However, recognition of the environmental occurrence of this cyanotoxin may have been constrained by the lack of specific analytical procedures to distinguish it from synthetic pesticides (overcome by Devic et al. 2002) and of the continuing unavailability of purified anatoxin-a(S).

24.3.6 Saxitoxins

The saxitoxins (Fig. 24.2), are a group of about 30 neurotoxic alkaloids which continue to be intensively studied in marine ecosystems (Llewellyn 2006, 2009; Llewellyn et al. 2006). They are highly potent (0.5–1 mg of pure saxitoxin constitutes a lethal dose for an adult human) and are produced by at least eight species of marine dinoflagellates. Evidence for saxitoxin production by marine macroalgae (*Jania*) also exists and it is possible that saxitoxins, or toxicologically-related metabolites, are produced by marine bacteria, including free-living species and bacterial symbionts associated with dinoflagellates (Llewellyn et al. 2006). The common name Paralytic Shellfish Toxins for these toxins is applied, since human illness and deaths have been caused by the consumption of shellfish contaminated after filter-feeding on marine dinoflagellates. Saxitoxins block voltage-gated sodium channels in excitable membranes by reversible binding to specific regions of the saxiphilin protein of the outer pore loops of the sodium channels (Bricelj et al. 2005; Llewellyn 2006, 2009). The resulting blockage of voltage-gated sodium channels inhibits the generation of functional action potential in nerves and muscle fibres and can rapidly (minutes) lead to paralysis and death by respiratory arrest. About 20 of the saxitoxin analogues have been identified in strains and environmental samples of the fresh- and transient-water genera *Anabaena*, *Aphanizomenon*, *Planktothrix*,

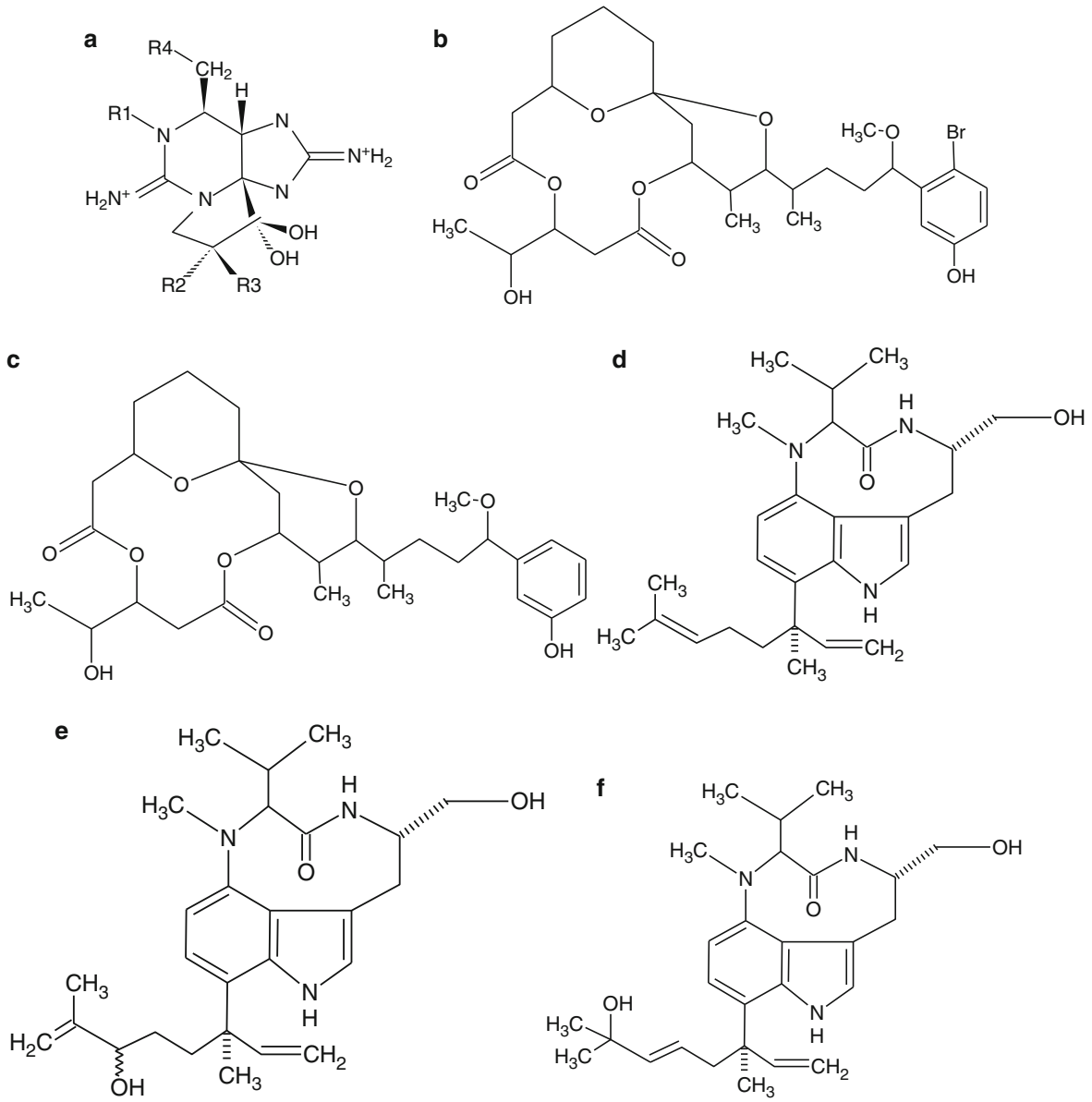

Fig. 24.2 Neurotoxins and biologically active cyanobacterial compounds. (**a**) Saxitoxins; (**b**) aplysiatoxin; (**c**) debromoaplysiatoxin; (**d**) lyngbyatoxin A; (**e**) lyngbyatoxin B; (**f**) lyngbyatoxin C

Cylindrospermopsis and *Lyngbya.* Saxitoxins are classified as Scheduled Chemical Weapons (Metcalf et al. 2006a; Metcalf and Codd 2009).

24.3.7 Lipopolysaccharides (LPS)

Although not in the high toxicity bracket alongside the previous cyanotoxins, there are increasing grounds to include LPS in the cyanotoxin checklist. A characteristic of the cyanobacteria, as with other Gram negative bacteria, is the presence of LPS in the outer membrane of the cell envelope (Drews and Weckesser 1982). The structure of LPS is that of a core oligosaccharide, an O-polysaccharide (outermost) component and an innermost lipid A region (Erridge et al. 2002). The highly hydrophobic lipid A moiety of LPS is primarily responsible for LPS toxicity, including in humans: hypertension, inflammatory responses and gastrointestinal upset. If untreated in susceptible humans, exposure to LPS from some of the *Enterobacteriaceae,* including *Salmonella* spp., may result in death. High variation occurs in lipid A composition between cyanobacterial species and strains of the same species, and between cyanobacteria and enteric bacteria (Drews and Weckesser 1982; Raziuddin et al. 1983; Martin et al. 1989). These differences in the toxicity-conferring component merit toxicity assessment of cyanobacterial LPS. The few *in vivo* toxicity determinations with purified cyanobacterial LPS conducted have indicated low toxicity

(Drews and Weckesser 1982; Raziuddin et al. 1983). However other necessary information on cyanobacterial LPS-exposure levels in relevant scenarios, a necessary part of LPS risk assessment, is largely lacking. The case for retaining LPS in the cyanotoxin checklist at present includes ongoing examples of human health problems associated with cyanobacterial mass populations (Sect. 24.4.2) and interactions between LPS and microcystins in increasing toxicity outcomes in fish; (Best et al. 2002). In addition to the case for including cyanobacterial LPS in cyanobacterial bloom toxicity assessment, the contribution of LPS from other Gram negative sources needs to be taken into account, since cyanobacterial mass populations in natural and controlled aquatic environments are invariably associated with other Gram negative prokaryotes.

24.3.8 Aplysiatoxins and Lyngbyatoxins

Through the pioneering work of Richard Moore and colleagues, research on these toxins was among the first to be carried out on cyanotoxins in marine environments. The phenolic bislactones aplysiatoxin and debromaplysiatoxin (Fig. 24.2) were identified as causative agents of "Swimmers' Itch", off Hawaii and Okinawa. The acute contact dermatitis involves severe cutaneous inflammation with blistering, erythema and desquamation. Aplysiatoxin is produced by strains of *Lyngbya majuscula* and the similarly toxic debromoaplysiatoxin is produced by *Schizothrix calcicola* and *Oscillatoria nigroviridis*. The indole alkaloid lyngbyatoxins-A, -B and -C (Fig. 24.2) are similarly dermatotoxic and are also produced by *L. majuscula* (Moore et al. 1993). Both cyanotoxin classes are protein kinase C activators, and thereby tumour-promoters (van Appeldoorn et al. 2007; Smith et al. 2008). Whether aplysiatoxin and debromoaplysiatoxin are exclusively cyanobacterial products is not clear. In animal intoxications by extracts of the red alga *Gracilaria coronopifolia*, the toxins may originate from the rhodophyte or from the growth of cyanobacterial endophytes (Ito and Nagai 1998, 2000).

24.3.9 Excitotoxic and Neurotoxic Amino Acids

More than 40 years ago, the excitotoxic amino acid β-*N*-methylamino-L-alanine (BMAA) was extracted and characterised from cycad seeds (Vega and Bell 1967). Studies have indicated that BMAA in *Cycas micronesica* tissues originates from the symbiotic *Nostoc* growing in the cycad's coralloid roots (Cox et al. 2003, 2005). Subsequent analyses indicated the presence of BMAA in *Nostoc* isolates obtained from seven out of ten symbioses namely the lichen *Peltigera*, the hornwort *Anthoceros*, the cycads *Cycas* and *Encephalartos*, and three spp. of the flowering plant *Gunnera*. Further analyses have indicated the presence of BMAA in a wide range of free-living cyanobacterial isolates and bloom samples examined from freshwater, marine and terrestrial sources (Cox et al. 2005, 2009; Banack et al. 2007; Esterhuizen and Downing 2008; Metcalf et al. 2008; Faassen et al. 2009; Craighead et al. 2009). These have included monocyanobacterial axenic isolates e.g. *Leptolyngbya* PCC 73110, with BMAA having been identified by multiple physicochemical methods (Spácil et al. 2010; Banack et al. 2007). Another neurotoxic amino acid, 2,4-diaminobutyric acid (DAB), has been reported in *Calothrix* PCC 7103 (Rosén and Hellenäs 2008), *Leptolyngbya* PCC 73110 (Spácil et al. 2010), further cyanobacterial isolates (Krüger et al. 2010) and, together with BMAA, in environmental samples from desert environments (Craighead et al. 2009; Cox et al. 2009). Research on the production, properties and fates of these amino acid neurotoxins is proceeding apace, not least because of the possibility that BMAA may be among the environmental causative agents of human neurodegenerative disease (Cox et al. 2003; Ince and Codd 2005; Pablo et al. 2009).

Table 24.2 Discovery and characterisation of cyanobacterial secondary metabolites: selected reviews

Osborne et al. (2001)	Tan (2007)
Harrigan and Goetz (2002)	Van Wagoner et al. (2007)
Watson (2003)	Berry et al. (2008)
Thajuddin and Subramanian (2005)	Gademann and Portmann (2008)
Dahms et al. (2006)	Gademann and Kobylinska (2009)
Welker and von Döhren (2006)	Jaiswal et al. (2008)
Wulff (2006)	Paul and Ritson-Williams (2008)
Bowling et al. (2007)	Smith et al. (2008)
Erpenbeck and van Soest (2007)	Wase and Wright (2008)
Leflaive and Ten-Hage (2007)	Lemmens-Gruber et al. (2009)

24.3.10 Other Cyanobacterial Products with Biological Activity

Cyanobacteria present a wide array of secondary metabolites, many with biotechnological and therapeutic potential. The approach which has usually led to their discovery, whether from environmental material, monocyanobacterial – (but bacterised) or, less often – axenic strains, has almost invariably been based on screening for particular biological activity, e.g. as an antimicrobial, or enzyme inhibitor, accompanied by the purification and structural characterisation of metabolites in the bioactive fraction. This quest usually begins with the preparation of solvent extracts from lyophilised material and continues to yield novel products. Over 20 reviews including bioactive cyanobacterial metabolites have appeared (Table 24.2) since these were considered by Dow and Swoboda (2000). The range of chemical structures includes small monocyclic and linear peptides (aeruginosins,

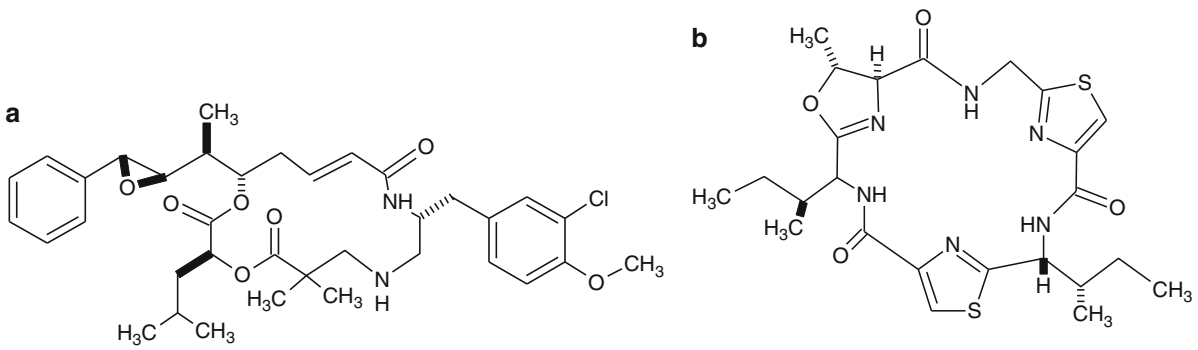

Fig. 24.3 The cyanobacterial bioactive products, (**a**) cryptophycin 52 and (**b**) aerucyclamide B

anabaenopeptins, cyanopeptolins, cyclamides, microginins) and multicyclic peptides (microviridins) (e.g. Harada et al. 1995; Welker and von Döhren 2006) and many linear lipopeptides (Tan 2007) e.g. malyngamides (Gerwick et al. 2001), spiroidesin (Kaya et al. 2002) and cyclostatins (Sano et al. 2005).

Knowledge of the biological activity, in contrast to the chemical structure, of many of these metabolites, is fragmentary and few studies have been carried out to evaluate activity against biota. Traditional screening and bioassay procedures against cell and enzyme targets has revealed antiviral, antibacterial, antifungal, antialgal, antiprotozoal, insecticidal activities and the inhibition of a range of proteases (see Table 24.2). The influence of the choice of screening method on the future direction of research is well exemplified by the history of cryptophycin. This depsipeptide from *Nostoc* was originally isolated as a fungicide, discarded as being too toxic, then re-isolated in a screen for cytoxicity using tumour cell lines with an IC_{50} of 50 pM (see Gademann and Portmann 2008). Cryptophycin 52 (Fig. 24.3a), one of the most potent and stable of over 25 analogues subsequently examined, was approved in Phase I clinical studies by Eli Lilly and proceeded to phase II studies involving human lung cancer patients. Although there was evidence of disease stabilisation, a need to revise dosing schedules to reduce toxicity was also identified to permit further progress in cryptophycin development as an anticancer drug (Edelman et al. 2003).

The need for novel drugs for chemotherapy against malaria, one of the world's most prevalent waterborne and fatal infections, is acute. A wide range of alkaloid and cyclic peptide products, with selective toxicity to the protozoan malarial parasite *Plasmodium falciparum* and related species, is emerging in studies with products from marine freshwater cyanobacteria e.g. *Nostoc* spp., *Oscillatoria* spp., *Lyngbya* spp. and *Microcystis aeruginosa*. Aerucyclamide B from *M. aeruginosa* (Fig. 24.3b) shows encouraging antiplasmodium selectively compared to a rat myoblast cell line (Gademann and Kobylinska 2009).

Modern systems biology-based strategies for drug discovery and development, combining in-silico analysis for drug target identification with high-throughput genomics and proteomics data, and receptor-ligand docking modelling are being usefully applied in the characterisation and application of cyanobacterial secondary metabolites (Wase and Wright 2008). This productive approach will complement, but not entirely replace, the continuing need for toxicity bioassays and ecotoxicological assessment methods in the detection and recognition of cyanotoxins (Depledge 2009).

24.4 Poisoning Incidents Associated with Cyanotoxins

24.4.1 Animal Poisonings

Much of our understanding of the ecotoxicology of cyanotoxins has been obtained through the investigation of animal and human health incidents. Post-event analyses have been performed, though usually to an incomplete degree, on cyanobacterial scum, bloom and mat samples, and on post-mortem material from actual and suspected poisoning cases involving a wide range of vertebrates, including humans. With the analysis of tissues, although cyanotoxins are not always absolutely confirmed as the cause, they are often considered to be the proximal and most likely cause. This can be from identification of cyanobacterial colonies and filaments in stomach and intestinal contents, or through the analysis of target organs such as the liver for microcystins. In the majority of cases, intoxication is accidental or incidental e.g. during drinking, although with some species which feed on cyanobacteria as their main or sole food source, intoxication due to cyanotoxins may occur as part of the life cycle at population level. Fish-kills may occur as a function of the normal life cycle of cyanobacterial blooms, in addition to their ability to produce cyanotoxins. For example, the decomposition of cyanobacterial blooms can reduce oxygen concentrations in the water resulting in the death of fish through asphyxiation.

The first recorded experimental investigation into the cause of animal deaths in which cyanobacterial blooms were

suspected was carried out by Francis (1878) after the deaths of livestock at Lake Alexandrina in South Australia. An extensive bloom of *Nodularia spumigena* had occurred and healthy sheep used in toxicity tests succumbed to the action of an unknown agent (presumably nodularin) after oral dosing with scum samples. The approach taken by Francis (1878) provided a model for subsequent investigations into cyanobacterial intoxications, long before the molecular identification of the agents proximally responsible (Codd et al. 1994).

Now that molecular methods for the identification of cyanotoxins and their genes are available, it is possible to apply these to ancient materials to investigate the possible presence and contributions of cyanotoxins. For example, investigations have inferred that Pleistocene mammals may have died due to ingesting microcystins (Braun and Pfeiffer 2002). Microcystin genes appear to have originated early in the evolution of cyanobacteria with sporadic and periodic gene loss (Rantala et al. 2004) and it is possible that cyanotoxins may have contributed to dinosaur mass mortalities in the Cretaceous Period (Varricchio 1995).

Francis (1878) examined the survival times and observed the stomach contents of the livestock, found cyanobacterial filaments within, and concluded that they were the cause of the poisoning and death. He also dosed sheep with the *Nodularia* scum to see if the signs of poisoning could be reproduced (as indeed they were). A wide range of animal species has subsequently been shown to succumb to cyanotoxins, including sheep, cattle, horses, pigs, primates, fish, bats and birds (Codd et al. 2005b, c). Dogs can be particularly susceptible to cyanotoxins and have died after ingesting planktonic cyanobacteria e.g. as scum biomass, and also benthic biomass e.g. wet mats and dried crusts of benthic cyanobacteria e.g. *Phormidium* containing anatoxin-a (Edwards et al. 1992). It has been considered that the musty taste and odour compounds often produced by cyanobacteria, including geosmin and 2-methylisoborneol, may render such biomass attractive to dogs through their scavenging habits. This may contribute to the circumstances at the edges of waterbodies where dog deaths have been investigated and found to include cyanotoxins as the major –, if not sole – cause of deaths (Codd et al. 1992). A further compounding factor for animals with fur coats is that immersion in water containing toxic cyanobacteria results in the adhesion of cyanobacterial filaments and colonies to the animal's fur. When the animals self-clean they are able to receive a further dose of cyanobacteria and the associated toxins. In the case of pets and farm livestock, it is advisable to minimise their exposure to water containing cyanobacteria, as with the rapid action of certain cyanotoxins in sufficient doses, animals often die before veterinary intervention can occur.

Cattle are also susceptible to cyanotoxin poisoning, due in part to the fact that they are often reared near to water sources which are nutrient-enriched and also because they consume large quantities of water. Cattle deaths attributed to microcystins and to a lesser degree anatoxins, have been reported from many countries, in what may initially seem to be unlikely scenarios e.g. high altitude Alpine summer lakes supporting benthic cyanobacteria (Mez et al. 1997). Cattle deaths have also occurred after ingesting *Cylindrospermopsis raciborskii* which contained cylindrospermopsin (Saker et al. 1999).

The above examples are viewed as accidental, or incidental, in the sense that the animals were exposed to the toxins during drinking. However microalgae and cyanobacteria are among the particulate biomass which serves as food sources for the filter-feeding, six species of flamingo known throughout the world. The Lesser Flamingo (*Phoeniconaias minor*), for example, feeds on *Arthrospira fusiformis* in the alkaline saline African Rift Valley Lakes. An endangered species, *P. minor*, is considered "Near Threatened" in the 2008 International Conservation Union (IUCN) red species list (Childress et al. 2008) and undergoes periodic mass mortalities, sometimes in the hundreds of thousands. Although a wide range of causes has been proposed for the mass die-offs, including a *Mycobacterium avium*-related epizootic, pesticides, heavy metals and (earlier) disturbance due to tourist pressures, analysis of Lesser Flamingo liver, stomach and intestine contents from flamingo carcasses identified significant concentrations of microcystins and anatoxin-a (Kotut et al. 2010; Ballot et al. 2003, 2004; Codd et al. 2003; Krienitz et al. 2003). Whether the body burdens of anatoxin-a and microcystins in the flamingos would have been sufficient to cause death as sole cause(s) is uncertain: cyanotoxin toxicity data are unavailable for this species. However, we infer that the cyanotoxins were likely to have been major contributors alongside the other (anthropogenic) insults (Krienitz et al. 2003). Mass mortalities of Greater Flamingo chicks (*Phoenicopterus ruber*) and of Chilean Flamingo (*Phoenicopterus chilensis*) adults at the Doñana National Park (a wetland) Spain, and Sea World, Orlando Florida USA, respectively have occurred, with deaths attributed to the ingestion of microcystins (Codd et al. 2003). An association of microcystin-producing *Microcystis* with the deaths of wild animals (White Rhinoceros, zebra, wildebeest) after ingestion of the bloom in Kruger National Park, South Africa has also been considered (Oberholster et al. 2009a).

24.4.2 Human Poisonings

Although less frequently reported and presumably less common than animal intoxications, human health incidents associated with cyanotoxins continue to be documented (Codd et al. 2005b, c). The most widely reported human health incident attributed to cyanotoxins occurred at a haemodialysis clinic in Caruaru in NE Brazil. The water to be treated for use in the clinic was tankered from a local reservoir containing cyanobacterial blooms. The clinic's water treatment procedure

was insufficient to remove cyanotoxins and microcystins remained in the water used for haemodialysis. Of the 116 people that experienced visual disturbances, nausea and vomiting, 76 patients died. The finding of microcystins in liver and serum samples indicated that 52 victims died as a result of cyanotoxins (Pouria et al. 1998; Jochimsen et al. 1998; Carmichael et al. 2001). Further analysis of filters used at the haemodialysis clinic at a later date identified cylindrospermopsin as a further cyanotoxin potentially involved in this event (Carmichael et al. 2001).

Human health effects through recreational exposure to microcystins have also occurred, most notably through water-based activities. Ten soldiers were affected with two being hospitalised after performing canoe submersion and swimming exercises at Rudyard Reservoir, Staffordshire, England. Cases of atypical pneumonia with blistering to the mouth and nasal passages after ingestion of *Microcystis aeruginosa* scum containing microcystins were reported (Turner et al. 1990). A similar case was recently reported in Argentina (Giannuzzi et al. 2011). Although not unequivocably identified as causative agents, cyanobacteria, including cyanobacterial LPS, were implicated in large-scale gastrointestinal (GI) upsets in 1975 at Selwickley, Pa, USA and in GI cases and mortalities in 1988 from the ingestion of water from the Itaparica Dam, Brazil (Lippy and Erb 1976; Teixeira et al. 1993).

An outbreak of hepatic enteritis occurred on Palm Island, Queensland Australia, among Aboriginal people. Although a number of causes were proposed, the drinking water source was found to be the likely site of the cause of the outbreak. From the Solomon Dam, the drinking water source for these people, a strain of *Cylindrospermopsis raciborskii* was isolated and cylindrospermopsin was extracted and identified with this cyanotoxin now considered to be the most likely cause of the "Palm Island Mystery Disease". For details of these outbreaks and of further outcomes of short-term exposures of humans to cyanotoxins, see Codd et al. (1999a, 2005b), Hilborn et al. (2007) and Humpage (2008).

Although the known human and animal health incidents attributed to cyanobacteria are generally short-term, increasing evidence indicates that cyanotoxins also have long-term human health implications. A study in China investigated the incidence of human primary liver cancer and found that the incidence was higher amongst those that drank from surface waters (containing microcystins) compared to neighbouring people who drank from deep wells with no cyanobacteria. The conclusion was that alfatoxins within the diet were initiating tumours and that microcystins were subsequently promoting tumour development (Ueno et al. 1996), a hypothesis consistent with tumour promotion by microcystins in rat oral-dosing studies (Nishiwaki-Matsushima et al. 1992). Further indication that long-term exposure to microcystins via (inadequately treated) drinking water is associated with human primary liver cancer has emerged from a large study around reservoirs in Serbia (Svircev et al. 2009). As the understanding and awareness of cyanotoxins and their health effects increases, further epidemiological studies need to be performed to inform, develop and promote health protection and guidelines and/or legislation worldwide. Certainly, in the case of an unusually high incidence of a neurodegenerative disease on Guam, cyanobacteria have been implicated as a potential causative agent through the production of BMAA decades after studies of the Chamorro people and ALS/PDC commenced. Recent research in New Hampshire, USA has found clusters of ALS patients around lakes known to have a history of cyanobacterial blooms at rates 10–25 times greater than the worldwide average incidence of this disease, although at present the reason for this is unclear (Caller et al. 2009).

24.5 Cyanotoxin Exposure Routes and Exposure Media

The understanding and reduction of animal- and human-intoxications and other adverse health outcomes by cyanotoxins require recognition of the exposure routes and exposure media involved. The currently perceived routes and media are summarised in Table 24.3. Understanding of the significance of exposure to airborne cyanotoxins is at an early stage with few epidemiological studies having been performed (Cheng et al. 2007; Stewart et al. 2006).

The most commonly encountered exposure medium for cyanotoxins is likely to be water and drinking water is thought be the most accessible route for cyanotoxin exposure in humans. Drinking water treatment methods have been examined for cyanotoxin removal and these vary widely in their effectiveness. No single procedure can be recommended for the optimal removal of all cyanotoxins, although some generalisations are possible. If the cyanotoxins of interest are largely intracellular and the producer-cells in the raw water to be treated are structurally intact, then procedures which remove the cells (e.g. flocculation, filtration) should essentially remove all of the cyanotoxins. However if the producer-cells are undergoing lysis before water treatment or are ruptured during the treatment process (e.g. breakage of *Microcystis* by hydrostatic forces during pumping), then this may result in increasing extracellular release of cyanotoxins. The removal of dissolved cyanotoxins from the water phase is aided by process selection and optimisation (e.g. Rodríguez et al. 2007; Acero et al. 2008; Rositano et al. 2001; Ho and Newcombe 2007; Ho et al. 2008, 2012).

Cylindrospermopsin-producing blooms of *Cylindrospermopsis raciborskii* present a special problem in water treatment since these typically release a high proportion of their cylindrospermopsin into the water phase during growth (e.g. up to 70% of the total pool; Norris et al. 2001;

Table 24.3 Principal routes, media and activities potentially involved in the exposure of humans and animals to cyanotoxins (Summarised from: [a]Codd et al. 1999a; [b]Krienitz et al. 2003; [c]Rellán et al. 2009; [d]Cox et al. 2009)

Exposure route	Exposure media	At-risk activities
Oral	Water	Daily drinking of raw or inadequately-treated water. Incidental/accidental consumption (recreation; animal watering)
	Food	Consumption of fish, shellfish, crustacean, irrigated crop-plant foods. Ingestion via regular diet (flamingos)[b]
	Dietary supplements	Consumption of preparations if containing cyanotoxins[c]
Dermal	Water	Skin and mucosal contact with blooms, scums during recreation, work practices; abrasions may cause predisposition
Inhalation	Water, aerosols	Spray containing toxigenic cyanobacteria/cell extracts during recreation, showering, work practices
	Dust	Inhalation of dust from dried planktonic biomass, desert crusts; work practices, military deployment[d]
Haemodialysis	Water	Contact with inadequately-treated haemodialysis water

Metcalf et al. 2002a). Access to adequately treated water, or indeed to drinking water which has received any treatment, varies widely throughout the world and exposure to cyanobacteria and cyanotoxins in drinking water may, in some situations, be unavoidable. Aboriginal people in regions of Australia traditionally dug soaks within the ground next to water sources. This allowed the bank of the soak to act as a crude filter to obtain drinking water of greater quality than in the main waterbody. For example, sand filters do develop good biofilms which have shown an ability to degrade cyanotoxins as the water passes through the filter. With adequate monitoring and prevention, it is possible to minimise the risk of exposure to cyanotoxins. In order to combat this, water companies have introduced a number of measures to minimise the possibility of human exposure to cyanotoxins. These include: catchment management measures to attempt to lower nutrient inputs and thereby reduce cyanobacterial bloom populations; lake sediment treatments, such as digging out sediment or the use of sediment locking treatments, to make nutrients bio-unavailable for cyanobacteria; alternative water sources which can be switched at the water treatment plant in the event that one source has a harmful cyanobacterial bloom; and, in the case that cyanotoxins actually enter drinking water to exceed guideline levels, the provision of bottled drinking water to households. With the events that occurred in Caruaru and the poisoning of dialysis patients (Jochimsen et al. 1998; Pouria et al. 1998; Carmichael et al. 2001), hospitals should have facilities which should permit the production of clean water for intravenous use during periods where cyanotoxins are present within the source water.

After drinking water, recreational exposure and exposure through foodstuffs are most likely the next most-commonly encountered exposure media for cyanotoxins in humans. Although cyanobacterial dietary or health-food supplements, comprised largely of Spirulina (Arthrospira) or *Aphanizomenon*, can contain cyanotoxins (Rellán et al. 2009; Gantar and Svirčev 2008; Bruno et al. 2006), the majority of exposure to cyanotoxin-contaminated food will be through accidental ingestion. Blooms of *Nodularia spumigena* are common in some eutrophic brackish transitional waters and particularly in the Baltic Sea (Mazur-Marzec and Plínski 2009). These blooms often contain high concentrations of nodularin. Bivalve molluscs including *Mytilus edulis* have the ability to filter litres of water per day and as a result may concentrate *Nodularia* filaments within their tissues. *Mytilus* collected during a bloom of *Nodularia* in Australia were subsequently found to be toxic to mice and collection of mussels for human consumption during such blooms is not recommended (Falconer et al. 1992). Other examples of accidental contamination of foodstuffs include crop-spray irrigation. A commercial lettuce grower in the UK used a brick pond to store water which was used for crop-spray irrigation. The pond developed and supported a bloom of *Microcystis aeruginosa* which was subsequently deposited onto the salad crop during irrigation, resulting in microcystin contamination which was deemed to be of unacceptable risk to the general population if the lettuces were consumed (Codd et al. 1999b).

Water-based recreational activities have been implicated in adverse health outcomes in inland and coastal waters via activities including paddling, swimming, kayaking and other water-contact activities such as sail-boarding. The risks of such activities were clearly exemplified by the illnesses among army cadets after swimming and 360° kayak-rolling in water containing *Microcystis* scum during training exercises at Rudyard Lake in the UK (Turner et al. 1990). Other at-risk activities include showering at lakes using lake water containing cyanobacteria and cyanotoxins, including the possibility of skin irritation and the inhalation of cyanobacteria and their toxins in mists and spraywater. Studies with intranasal administration to mice of anatoxin-a and microcystins as sprays have shown that damage to mucus membranes is possible and that acute toxicity via inhalation can be at least as high as via oral administration (Fitzgeorge et al. 1994).

24.6 Persistence and Biological Processing of Cyanotoxins

Whole body studies have shown that, in addition to the accumulation of a proportion of the absorbed microcystin, enzyme-mediated detoxification can occur *in vivo*, with

elimination of a proportion of the microcystin and/or detoxified products in the urine and faeces (Falconer et al. 1986; Brooks and Codd 1987; Robinson et al. 1991). When organisms are exposed to cyanotoxins in acute doses the most likely outcome is death. Even when exposed at lower concentrations, the possibility exists that long-term health effects can occur, such as an increase in primary liver cancer considered to be caused by tumour initiation by aflatoxin and tumour promotion by microcystins (Ueno et al. 1996). However, research is beginning to show that cyanotoxins can be metabolised by microbes, and by invertebrates, vertebrates and plants, although the focus so far has been on microcystins and nodularin (Wiegand and Pflugmacher 2005).

Microcystins are chemically stable molecules. They can survive extended boiling (half-life, about 24 h) and are stable between pH1 and pH10 (Codd and Bell 1996). Boiling of acidic solutions of saxitoxins can create more toxic variants (Etheridge 2010) and although saxitoxins may be leached from seafood into the cooking water (Lawrence et al. 1994), the thermostability of these compounds may still present a risk to consumers (Stommel and Watters 2004; Etheridge 2010).

Although relatively resistant to chemical degradation, cyanotoxin susceptibility to biodegradation occurs. From studies with microcystins, bacteria have been isolated from fresh and brackish waters, often where cyanobacterial blooms are common, which can degrade microcystins and nodularin in a matter of hours to days. The bacteria include species/strains of *Sphingomonas*, *Sphingocinicella*, *Arthrobacter*, *Brevibacterium*, *Rhodococcus* and *Burkholderia* (Mazur-Marzec and Plínski 2009; Kato et al. 2009). The sequence of enzymic degradation of microcystins involves initial attack of the cyclic ring to create a linear peptide, followed by the degradation of the linearised peptide to a tetrapeptide, before final breakdown to Adda, which does not show toxicity and can persist in the aquatic environment for some time (Bourne et al. 1996; Imanishi et al. 2005). Consistent with the general principles of resource enrichment conditions for the selection of microbial activity, cylindrospermopsin-degrading activity was enhanced in *Cylindrospermopsis raciborskii* blooms (Smith et al. 2008), although this has not been observed with cylindrospermopsin-producing blooms of *Aphanizomenon ovalisporum*, despite toxin enrichment attempts (Wormer et al. 2008).

Whilst some bacteria can process cyanotoxins, diverse eukaryotes have mechanisms which minimize the potential toxicity. Initial investigations with rodents found that a proportion of the cyanotoxin administered was excreted in the urine and faeces, either as authentic cyanotoxin or as toxin-products (Falconer et al. 1986; Brooks and Codd 1987; Robinson et al. 1991) and these may be significant routes for cyanotoxin elimination from the mammalian body.

From exposure studies using cysts, nauplii and adults of the brine shrimp *Artemia salina*, glutathione-*S*-transferases were found to modify microcystins and nodularin (Beattie et al. 2003). Cyanotoxin enzymic conjugation products including glutathione-microcystin, cysteine-glycine-microcystin and cysteine-microcystin have been identified after incubation with glutathione S-tranferases from a wide range of plants and animals *in vitro* (Wiegand and Pflugmacher 2005) These products have all been found to have substantially lower toxicity (ca. 90% reduction) according to mouse bioassay and by protein phosphatase enzyme inhibition assay (Metcalf et al. 2000a), indicating a considerable capacity to detoxify microcystins and nodularin. The cytochrome P450 system has similarly been implicated as a potential detoxication system for cylindrospermopsin (Norris et al. 2002).

In addition to excretion and detoxication processes, other potential routes of cyanotoxin elimination, e.g. respiratory, or via saliva, sweat, milk, hair, skin, nails (hoofs, horns) and in the case of neurotoxins, cerebrospinal fluid (Hughes 1996) have received little attention. The Lesser Flamingo, *Phoeniconaias minor* has experienced periodic mass mortalities attributed, alongside other environmental toxicants, to cyanotoxins (Krienitz et al. 2003, 2005). However, considering the intimate association of cyanobacteria with the Lesser Flamingo, as a major and potentially sole food source, this bird may have developed multiple means of processing cyanotoxins. Analysis of feathers from *P. minor* found no significant adsorbtion of dissolved cyanotoxins from water. However microcystins and anatoxin-a were present in feathers taken from bird carcasses at Lakes Bogoria and Nakuru, Kenya (Metcalf et al. 2006b). These findings, consistent with the presence of the cyanotoxins in the gut contents and livers of dead birds, indicate that microcystins and anatoxin-a, after ingestion by flamingos, can be eliminated to some degree into their feathers. This may be an adaptation to life with potentially toxic foodstuffs. A similar scenario exists in the Baltic Sea where Eider Ducks (*Somateria mollisima*) feeding on juvenile mussels are potentially exposed to nodularin and this cyanotoxin has been found in the feathers of these birds (Sipiä et al. 2008).

As an increasing amount of evidence indicates that cyanotoxins can be processed by lower and higher animals, including mammals, it may be that poisoning incidents observed as severe illness or death, are the extreme examples of cyanotoxin exposure. Thus an array of excretion, detoxication and elimination mechanisms may be involved in reducing the likelihood of an adverse health outcome in an otherwise healthy and unchallenged individual. In the event of exposure to multiple toxic hazards, especially if these present demands on a common detoxication mechanism e.g. glutathione S-transferase, and if the subject is additionally compromised e.g. via physiological stress, then the balance between successful tolerance of exposure to cyanotoxins can be tipped towards an adverse outcome: illness or death.

24.7 Cyanotoxin Production

Initial studies on the production of cyanotoxins have focused on the supply and manipulation of nutrient concentrations and physical conditions during growth. Such studies have included effects of temperature, irradiance, cell density, nitrogen, phosphorus and iron supply, both under steady-state conditions in continuous culture and in batch culture. In most cases alterations in cyanotoxin concentration on a per cell basis were observed. However, in none of the cases was cyanotoxin production eliminated and differences in toxin concentrations have been limited to within a single order of magnitude e.g. between 2- and 5-fold (Sivonen and Jones 1999 and references therein; Wood et al. 2012).

Although such studies continue to be useful, more recent research has focused on the mechanisms by which cyanotoxins are synthesized. Initial studies of microcystin synthesis found that, like bacterial antibiotics including tyrocidin and Gramicidin S, microcystins are thiotemplate-, non-ribosomal and non-mRNA-dependent products (Arment and Carmichael 1996; Dittmann and Börner 2005; Kehr et al. 2011). Although cyclic peptides, this research indicated that microcystins are not ribosomally-produced peptides but are complex enzyme products. Earlier research into non-cyanobacterial, non-ribosomally-produced peptides, e.g. in *Bacillus* had identified genes and enzymes responsible for their biosynthesis (Marahiel et al. 1997; Dittmann and Börner 2005). By analogy, heterologous gene hybridization (e.g. Marahiel et al. 1997) and enzyme assays *in vitro*, similar methods were then applied to the production of cyanobacterial toxins. Peptide synthetases were found which when deleted using genetic manipulation resulted in the loss of microcystin synthesis from the cell (Dittmann et al. 1997). Further analysis of the genome indicated that the operon for the synthesis of microcystins was large, comprised of ten bidirectionally transcribed genes in *Microcystis* (Nishizawa et al. 1999, 2000; Tillett et al. 2000; Dittmann and Börner 2005). Species differences have also been reported in microcystin synthetase gene sequences, for example between *Microcystis* and *Planktothrix* (Christiansen et al. 2003). The microcystin genes have also proven useful for examining how long this product of cyanobacteria has existed and how often it has been lost. By studying microcystin synthetase sequences, Rantala et al. (2004) found that microcystin function was an ancient phenomenon that has periodically been lost. Although at present no absolute known function for the microcystins has been elucidated, genetic analysis may prove useful in this regard.

The identification of gene products and enzymes for the synthesis of microcystins has spurred research into the identification of synthetic systems for other cyanotoxins, including a gene cluster for nodularin (Moffitt and Neilan 2004). Cylindrospermopsin similarly has peptide synthetase and polyketide synthase components which have proven useful for the discrimination of cylindrospermopsin-producing and non-cylindrospermopsin-producing strains (Schembri et al. 2001). The other cyanobacterial toxin which is receiving increasing attention in terms of its genetic regulation are the saxitoxins and a biosynthetic gene cluster has been identified (Kellmann et al. 2008). Certainly, in the marine environment, saxitoxin production by dinoflagellates may have a bacterial component (Vásquez et al. 2001), but it is still unclear whether bacteria associated with cyanobacterial blooms may contribute to the concentrations reported from them.

24.8 Analytical Methods for Cyanotoxins

24.8.1 Range of Methods

Reliable, specific and sufficiently sensitive methods are required to detect and quantify cyanotoxins in natural and controlled environments. A wide range of extraction and analytical methods have been developed (e.g. Fig. 24.4), employing a variety of clean-up and detection systems. The systems developed and the concentrations at which analytical methods can detect toxins are driven by a number of factors. These include: (i) ethical considerations (in the case of toxin bioassays on animals); (ii) national legislation or guidelines (which influence the specification of minimum limits of quantification and of detection); (iii) mechanisms of toxicity; (iv) currently available technologies; (v) requirements to detect and quantify cyanotoxins in complex matrices, e.g. fish tissues, in addition to cyanobacterial cells and water, and (vi) the need to detect and quantify further emerging cyanotoxins. Furthermore, there is a now a trend towards the provision of easy-to-use methods which can be used away from centralised, specialist laboratories. However, the latter continue to be needed for qualitative and quantitative verification.

As the range of analytical methods is diverse, often the preparatory methods need to be tailored to the analytical methods used. This can involve the use of solvents for physicochemical methods through to aqueous extraction methods when immunoassays are generally employed. Furthermore, detection and confidence can be enhanced through the introduction of sample clean-up methods including solid phase extraction before the analysis to enable other compounds which may interfere with analysis to be removed. The following sections provide background on many of the analytical methods in current use for the preparation and analysis of samples, extracts and cyanotoxins in a range of systems and scenarios.

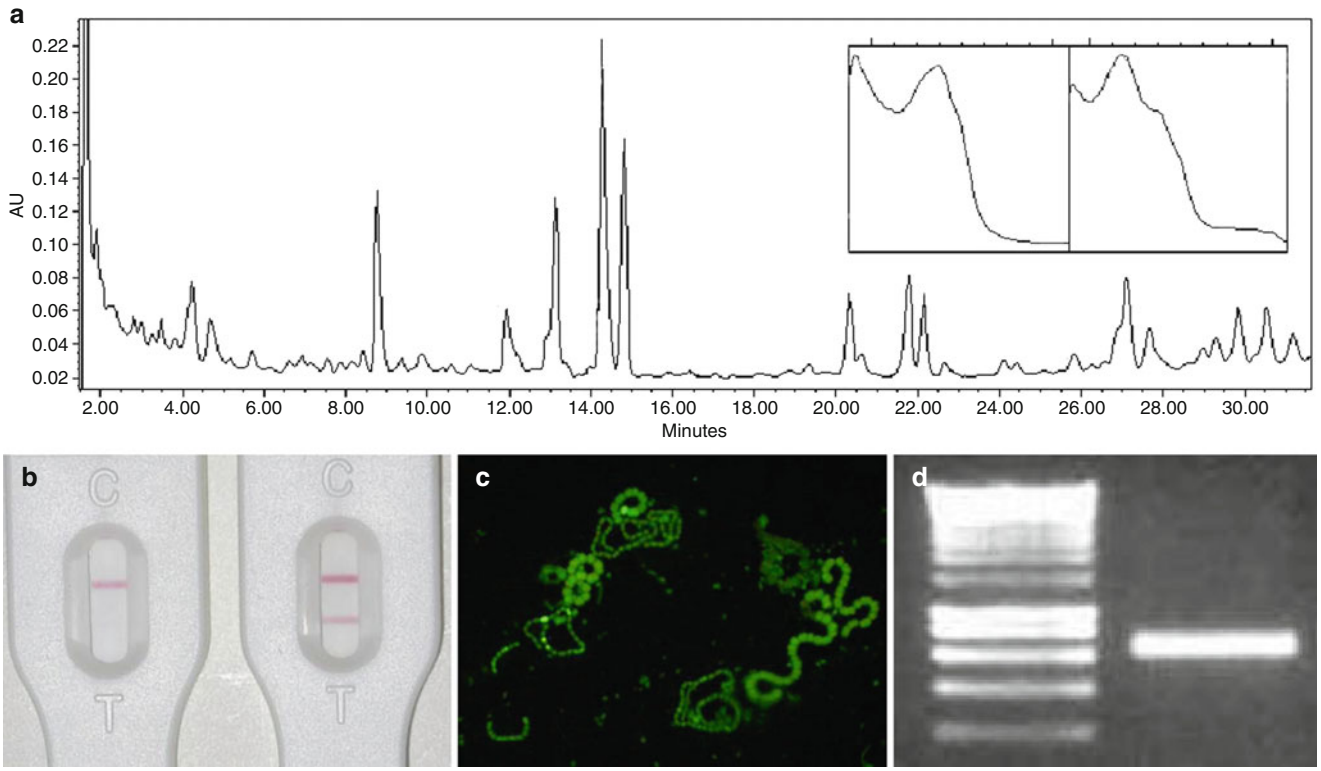

Fig. 24.4 Examples of analytical methods for cyanotoxins and genes related to their synthesis. (**a**) HPLC, with diode array detection of an extract of *Microcystis aeruginosa* showing inset spectra for MC-LR and MC-WR. (**b**) Lateral flow microcystin immunoassay showing negative sample (*left*) and positive sample (*right*). *Top line* in each cassette shows that the test has worked, while the presence of an additional *bottom test line* shows the presence of microcystins in the sample. (**c**) Fluorescence *in situ* hybridisation of microcystin synthetase DNA in *Anabaena* from a Scottish freshwater body. (**d**) Microcystin PCR showing DNA ladder (*left*) and amplified microcystin DNA from a cyanobacterium

24.8.2 Extraction and Sample Preparation Methods

For any analytical method, good extraction techniques are required to remove the maximal amount of cyanotoxins from the cells or other matrix of interest and, in advance of physico-chemical methods, to remove interfering compounds. Often, the extraction method may also be designed and refined and optimised for a particular analytical procedure. Methods of processing cyanobacterial bloom and scum samples may be to simply disintegrate the cells and release the cyanotoxins into the aquatic medium in which the cyanobacteria are present. This can be achieved by ultrasonication to disrupt the cells through microcavitation and heat or by boiling the sample for a short period e.g. 1 min. After cell disruption, the débris can be removed by filtration or bench centrifugation. These simple procedures are useful for the maximal release of microcystins from a wide range of planktonic and benthic cyanobacterial species (Metcalf and Codd 2000).

For most cyanobacterial preparations for analysis, further processing is often necessary. This is generally performed by concentrating the cellular material from the water or bloom sample e.g. by sedimentation, or in the case of positively buoyant material, by allowing the cyanobacterial colonies, bundles or gyres to rise in the sample vessel. After the recovery of partially concentrated biomass, this can be further concentrated by centrifugation, with gas vesicles being collapsed in advance by a few vigorous impacts of the closed plastic centrifuge tubes on the bench.

After the cyanobacterial cellular material has been obtained, this is usually frozen (e.g. at −20°C) and lyophilised. Frozen and lyophilised samples can be stored at −20°C for many years and still yield cyanotoxins upon analysis (Metcalf et al. 2008), although cyanotoxin losses after such long periods have not been quantified. After freeze-dried material has been prepared, extraction with solvents is often performed. For microcystins, 5% acetic acid in water and 70–75% methanol in water (Lawton et al. 1994; Fastner et al. 1998; Ward et al. 1997) have been successfully used. The presence of water in the extraction solvent has allowed some of the more hydrophobic microcystins, such as MC-LW and MC-LF to be more efficiently extracted, whilst still retaining good extraction efficiency for hydrophilic microcystins (Ward et al. 1997). For cyanotoxins such as anatoxin-a (Edwards et al. 1992) and anatoxin-a(S) (Devic et al. 2002), aqueous extraction is most commonly employed. Several

methods for cylindrospermopsin extraction have been examined, including water and methanol and of these 5% formic acid in water was best (Törökné et al. 2004). LPS can be extracted from cyanobacteria in a number of ways including hot phenol-water, proteinase K and EDTA. Of four methods compared, that involving Proteinase K, RNase and DNase results in minimum extraction time, maximum LPS yield and minimum co-extraction of microcystins (Lindsay et al. 2009). Saxitoxins are routinely extracted by aqueous or salt conditions (e.g. Molica et al. 2005).

Although optimised solvent extraction can subsequently give improved analytical results, cyanotoxins can often be present at low concentrations and may require concentration before analysis, and some extracts may still contain compounds that interfere with the analytical method. In order to clean-up and concentrate extracts, a number of solid phase extraction techniques have been developed. For microcystins, C18 solid phase extraction cartridges have proven very effective in decreasing detection limits and improving analysis (Lawton et al. 1994). Such cartridges are also useful for anatoxin-a, although for effective retention, a pH of 9.6 is required in the water or extract (Rapala et al. 1993). With improvements of solid phase extraction technology, other sorbents continue to be developed and of these graphite carbon (Norris et al. 2001; Metcalf et al. 2002a; Wormer et al. 2009) and polymeric sorbents (Kubo et al. 2005) show good retention of cylindrospermopsin. Recent interest in BMAA has also resulted in SPE methods which can isolate this cyanotoxin using strong cation-exchange resins (Kubo et al. 2008; Spácil et al. 2010; Jonasson et al. 2010).

With developments in antibody technology, immunoaffinity cartridges are being developed and these may provide even cleaner extracts for analysis and improved verification as a result of their specificity (Aranda-Rodriguez et al. 2003). These cartridges are now being applied to complex matrices including fish tissues (Kondo et al. 2005). With advances in technology and decreases in cost, immunoaffinity columns may become economically viable for the specific concentration and clean-up of microcystin-containing extracts.

24.8.3 Physicochemical Methods

24.8.3.1 Introduction

Physicochemical methods employ analytical machinery to separate and detect metabolites of interest, including cyanotoxins. When liquid chromatography is used, this can be by either normal phase or reverse phase, the latter being more commonly used when cyanotoxins are analysed. The range of detectors which can be used is vast and is assessed as to applicability. Reviews on the analysis of cyanotoxins by physicochemical methods include McElhiney and Lawton (2005), Meriluoto and Codd (2005) and Codd et al. (2001).

24.8.3.2 UV Spectroscopic Methods

Before analytical techniques can be used to identify and quantify cyanotoxins, defined cyanotoxin standards need to be produced to calibrate the equipment. Several methods can be used for the preparation of cyanotoxin standards of analytical quality, the majority relying upon cyanotoxin extraction and purification from laboratory-grown cyanobacterial strains or environmental bloom material. Once obtained, the purified cyanotoxin is characterised and quantified by a number of standard analytical chemistry techniques. Of particular importance is the need for molar extinction coefficients. Once obtained these can be used to quantify purified cyanotoxins under defined chemical conditions in a UV spectrophotometer to provide standards to calibrate further analytical equipment. Several extinction coefficients for cyanotoxins have been published (see Meriluoto and Codd 2005) and recently the extinction coefficient for cylindrospermopsin has been revised (Sano et al. 2008).

24.8.3.3 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) was one of the first mainstream physicochemical methods to be used for cyanotoxin analysis. It continues to be the most commonly used physicochemical method. The versatility of HPLC systems and their detectors have resulted in their ability to measure microcystins, nodularins, cylindrospermopsin, anatoxin-a and saxitoxins, as examples. Nodularins, microcystins, cylindrospermopsin and anatoxin-a are routinely determined by HPLC with either UV or photodiode array (PDA) detection (Codd et al. 2001; Meriluoto and Codd 2005). UV detectors at a single set wavelength can be used to compare retention time and peak area of a known standard with that of the analyte, but this has now been largely superseded by the PDA detector. The latter allows individual cyanotoxins to be detected at a specific UV wavelength and then subsequently scanned, usually from 200 to 300 nm, to give a defined spectrum for that compound. Microcystins (and nodularins), cylindrospermopsin and anatoxin-a all give characteristic spectra (Figs. 24.4a and 24.5) which, when combined with retention time, can provide useful identification and quantification of the cyanotoxin. The use of HPLC-PDA spectra, with subsequent mass spectrometry and structural determination, has led to the identification of the more than 90 microcystin variants currently known.

The nature of the solvents employed may also affect the subsequent response, as demonstrated with a cylindrospermopsin standard prepared in increasing methanol concentrations and analysed by HPLC-PDA (Metcalf et al. 2002a). HPLC methods for cyanotoxins continue to evolve, e.g. by application of novel separation columns. However, high capital equipment and operating costs and the need to provide

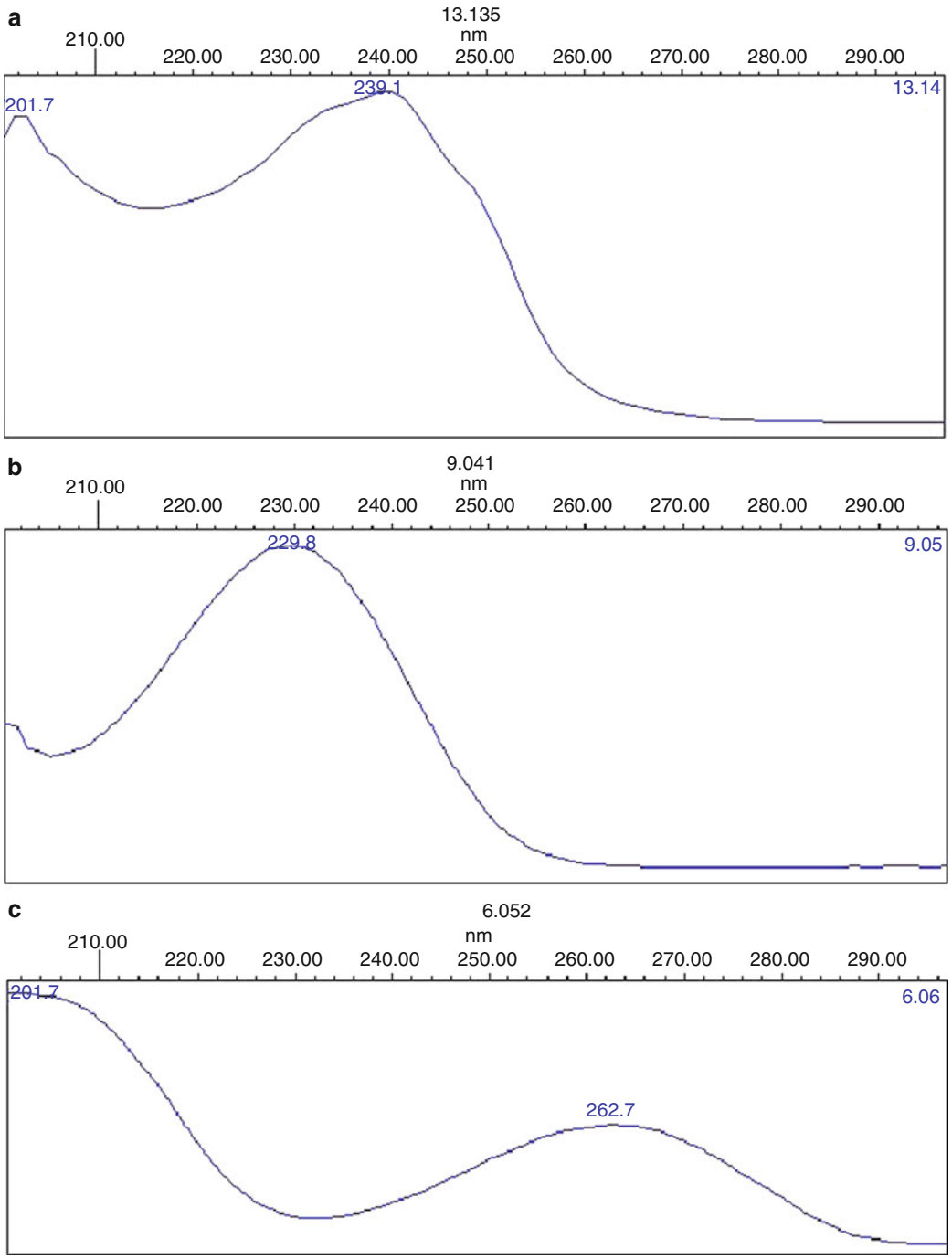

Fig. 24.5 Examples of PDA spectra for the cyanotoxins microcystin-LR, anatoxin-a and cylindrospermopsin. (**a**) Microcystin-LR; (**b**) anatoxin-a; (**c**) cylindrospermopsin

real-, or near-real-time data for water management, all increase the need to simplify and accelerate methods. These needs were recently (2008–2009) exaccerbated by a global shortage of acetonitrile, which is almost universally used as a solvent in cyanotoxin analysis by HPLC. This has required methods to be developed which avoid the need to use this solvent (e.g. Purdie et al. 2009).

Although many cyanotoxins contain UV chromophores which allow their detection by UV or PDA detectors, the saxitoxins require derivatisation to permit spectrometric detection. The resulting fluorescent oxidation products can be detected by an HPLC equipped with a fluorescence detector (Cianca et al. 2007). HPLC with fluorescence detection has also been employed for the measurement of BMAA, by

derivatisation with chloroformate (Montine et al. 2005; Esterhuizen and Downing 2008) and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (Cox et al. 2005; Banack et al. 2007).

Other technological advances in cyanotoxin analysis by HPLC, include the use of short and narrow HPLC columns for the rapid assessment of microcystins (Spoof et al. 2010) and the application of Ultra Performance Liquid Chromatography (UPLC). UPLC methods, with PDA detection have now been successfully applied to the analysis of microcystins, and this technology has the potential to increase speed of analysis and the sensitivity and resolution in comparison with traditional HPLC methods (Spoof et al. 2009).

24.8.3.4 Mass Spectrometric (MS) Methods

The molecular mass of a compound can also be used to identify cyanotoxins. Microcystins and nodularins have a molecular mass between approximately 800 and 1,200 Da; anatoxin-a has a molecular mass of 165 Da and cylindrospermopsin of 415 Da, as examples. Single quadrupole mass spectrometers can be useful when combined with the retention time of a compound in liquid chromatography and can determine the presence of a cyanotoxin. However, in the case of microcystins, as there are currently over 90 known variants, analysis using LC-MS/MS with the production of daughter ions, including 135 for the Adda portion (Edwards et al. 1993), can prove useful for verification and permit the identification of other microcystins for which reference materials might not be available. Cyanotoxins can also be measured by gas chromatography (GC) coupled to MS such as for the production of the oxidation product of Adda, erythro-2-methyl-3-methoxy-4-phenylbutyric acid (MMPB; Yuan et al. 2006). Not all MS determination of microcystins includes chromatography. Matrix-Assisted Laser Desorption Ionisation- Time of Flight (MALDI-TOF; Erhard et al. 1997) and Surface Enhanced Laser Desorption Ionisation-Time of Flight (SELDI-TOF; Yuan and Carmichael 2004) have also proven useful for the determination of microcystins and a wide range of related cyanobacterial metabolites. Other advances for the rapid LC-MS separation and analysis of microcystins have included the introduction of UPLC using MALDI-TOF detection (Wang et al. 2007; Ortelli et al. 2008). Recently, MS using Hydrophobic Interaction Liquid Chromatography (HILIC) has been applied to the detection of anatoxin-a(S), microcystins, anatoxin-a, saxitoxins and cylindrospermopsin (Dörr et al. 2010; Dell'Aversano et al. 2004).

24.8.3.5 Nuclear Magnetic Resonance (NMR)

Although not commonly used for cyanotoxins, NMR has detailed the structures of several cyanotoxins, including microcystins (Schripsema and Dagnino 2002).

24.8.4 Biochemical and Biological Methods

24.8.4.1 Bioassays, Enzyme Assays and Immunoassays

The original toxicity assessment method, bioassay involving susceptible test organisms, has been and continues to be extremely useful for the toxicity assessment of cyanobacterial whole cells, lysates, crude extracts and (partially) purified compounds. Since, Francis (1878) observed dead sheep along the Murray Darling River and experimentally dosed healthy sheep with *Nodularia spumigena* scum to identify the cause of death, animals have been used for cyanotoxin bioassays. Bioassays are invaluable in guiding the fractionation procedure to focus on individual fractions thereby leading to the eventual isolation of individual novel products. A heavy reliance upon the use of mice, rats and pigs, the latter due to their similarities in physiology to humans, has provided data on the modes of action of cyanotoxins, resulting pathology and quantitative susceptibilities to administration via the relevant exposure routes (e.g. Falconer et al. 1994; Metcalf et al. 2000a; Humpage 2008). However, due to ethical considerations concerning bioassays with vertebrates, invertebrates are increasingly being used to assess cyanobacterial bloom toxicity and that of cyanobacterial extracts. *Daphnia* spp. and the brine shrimp *Artemia salina* have been increasingly used as statistical analysis can be more easily applied, and the invertebrates can show similar sensitivities to cyanobacterial bloom extracts as the vertebrates (Lawton et al. 1994; Metcalf et al. 2002b). Further screening of potential bioassays with aquatic biota identified the crustacean *Thamnocephalus platyurus*, widely used in aquatic ecotoxicology, as a sensitive bioindicator of microcystins (Tarczynska et al. 2001; Drobniewska et al. 2004). Assays with *A. salina* are also being applied to assess combinations of cyanotoxins, both via simultaneous and sequential exposure, neglected factors which greatly influence toxicity outcomes (Lindsay et al. 2006). Plants are also increasingly used for the assessment of cyanotoxins and have been successfully used as a bioassay for microcystins (McElhiney and Lawton 2005; Oberholster et al. 2009b) and cylindrospermopsin (Vasas et al. 2002; Metcalf et al. 2004).

Whilst bioassays are useful for the initial screening of environmental material for toxicity, and may be essential in the absence of major capital equipment, they do not provide specific identification of the cyanotoxins involved. However, where molecular targets have been elucidated these can be used in biochemical assays for cyanotoxin detection and quantification. These include protein phosphatases for microcystins and nodularins (MacKintosh et al. 1990), sodium channels and saxiphilins for saxitoxins (Mahar et al. 1991), acetylcholine receptors for anatoxin-a (Aráoz et al. 2005, 2008), protein translation for

cylindrospermopsin (Froscio et al. 2001) and engineered acetylcholine esterases for anatoxin-a(S) (Devic et al. 2002). Increasingly, biochemical assays for cyanotoxins are being combined with other procedures, such as the combination of SPE with protein phosphatase inhibition assay (Rivasseau et al. 1999) and the combination of microcystin antibodies with the protein phosphatase inhibition assay (Metcalf et al. 2001). The protein phosphatase inhibition assay also has the potential to be developed as a biosensor (Allum et al. 2008). LPS concentrations can be determined using the *Limulus polyphemus* gel clot and lysate assays (Lindsay et al. 2009) using colorimetric substrates and spiked controls. Due to the variable structure of LPS, gel electrophoresis methods have proven extremely useful, although rapid analysis and risk assessment using such methods is difficult.

Antibodies against cyanotoxins have revolutionised the ability to measure certain cyanotoxins in a range of environments and materials (Metcalf and Codd 2003). Antibodies have been produced against microcystin-LA (Kfir et al. 1986), microcystin-LR (Chu et al. 1989; Metcalf et al. 2000b), microcystin-RR (Young et al. 2006), nodularin (Mikhailov et al. 2001), the Adda fragment of microcystins and nodularin (Fischer et al. 2001), saxitoxins (Chu et al. 1992) and cylindrospermopsin (Bláhová et al. 2009). Although these antibodies have been developed as lab-based enzyme-linked immunosorbent assays (ELISA; e.g. Metcalf et al. 2000b; Khreich et al. 2009), increasingly antibody-based technologies for cyanotoxins are being developed for rapid, easy-to-use devices in the field through the development of immunostrips (Lawton et al. 2010; Tippkötter et al. 2009; Fig. 24.4b).

24.8.4.2 Genetic Methods to Assess Cyanotoxin-Producing Potential

The majority of analytical methods for cyanotoxins measure the analyte of interest. However, in some cases the potential for cyanotoxin production can be assessed using genetic methods. Polymerase chain reaction (PCR) methods have been developed for microcystin synthetases (e.g. Rantala et al. 2004; Fig. 24.4d) and genes required for cylindrospermopsin production (Schembri et al. 2001). These can be used to assess whether cyanobacterial blooms samples and individual bloom members, after isolation, have microcystin- or cylindropsermopsin-producing potential. Genetic methods may also be useful for the direct microscopic assessment of mixed populations of cyanobacteria by fluorescence *in situ* hybridisation (FISH; Metcalf et al. 2009; Fig. 24.4c). The ability to measure RNA by reverse transcription QPCR has also recently been applied to the detection of microcystin gene transcripts (Ruecekert and Cary 2009).

24.9 Co-occurrence of Multiple Cyanotoxins and Other Health Hazards in Cyanobacterial Blooms

Research into the ecotoxicology of cyanobacteria has largely focused either on bioassays in which individual cyanotoxins cannot be identified (see Sect. 24.8.4.1), or on the environmental occurrence and specific properties of an individual cyanotoxin or cyanotoxin family. Analyses for multiple classes of cyanotoxins have been relatively rare. Furthermore, the environmental and health significance of combinations of known cyanotoxins, particularly from the different classes with different modes of action (Sect. 24.3), has hardly been investigated. However the production of multiple classes of cyanotoxins appears to occur throughout the full range of genotype and phenotype combinations in which cyanobacteria can be encountered. These range from: (i) production of multiple microcystins plus anatoxin-a(S) by individual cultured strains of *Anabaena flos-aquae* (Matsunaga et al. 1989; Harada et al. 1991) to: (ii) combinations of microcystins plus anatoxin-a in mixed cyanobacterial blooms in African Rift Valley lakes (Krienitz et al. 2003, 2005): (iii) nodularins plus microcystins in northern and southern regions of the Baltic Sea (Codd et al. 2005b; Kankaanpää et al. 2009; Mazur-Marzec et al. 2008). Since LPS appears to be a universal component of the cyanobacterial cell envelope, this endotoxin at least can be assumed to invariably co-occur with other cyanotoxins (Codd et al. 2005b). Whilst the extent of BMAA and DAB production by cyanobacterial laboratory strains and natural populations is still under investigation, examples of the co-occurrence of BMAA with microcystins, cylindrospermopsin, nodularin and anatoxin-a are available (Metcalf et al. 2008; Cox et al. 2005). It is also assumed that BMAA and DAB co-occur with cyanobacterial LPS, in addition to the LPS of any other associated Gram negative prokaryotes in natural cyanobacterial populations or non-axenic laboratory cultures.

In addition to the potential for cyanobacteria to produce multiple cyanotoxins which can affect human and animal health, environmental populations of cyanobacteria are seen as nutritional resources and refuges for aquatic microbial pathogens. The greatly extended viability of *Vibrio cholerae* in the mucilage sheath of *Anabaena* gyres in Bangladeshi waters in regions with cholera epidemics raises the possibility that cyanobacterial blooms may promote the persistence of cholera in such environments (Islam et al. 1994; Colwell 1996). This also applies to cyanobacteria in biofilms (Islam et al. 2007). There is a further possibility that algal and cyanobacterial blooms may provide a refuge for pathogenic (Shiga toxin-producing) *Escherichia coli* of human faecal origin (Ishii and Sadowsky 2008). With increasing human

pressures in many parts of the world on dwindling water resources, for potable supply and sewage disposal, the health significance of cyanobacterial blooms may thus extend beyond the currently known cyanotoxins.

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References

Aboal M, Ángeles Puig M, Asencio AD (2005) Production of microcystins in calcareous Mediterranean streams: the Alharabe River, Segura River basin in south-east Spain. J Appl Phycol 17:231–243

Acero JL, Rodríguez E, Majado ME, Sordo A, Meriluoto J (2008) Oxidation of microcystin-LR with chlorine and permanganate during drinking water treatment. J Water Supply Res Technol AQUA 57:371–380

Allum LL, Mountfort DO, Gooneratne R, Pasco N, Goussain G, Hall EAH (2008) Assessment of protein phosphatase in a re-usable rapid assay format in detecting microcystins and okadaic acid as a precursor to biosensor development. Toxicon 52:745–753

Aranda-Rodriguez R, Kubwabo C, Benoit FM (2003) Extraction of 15 microcystins and nodularin using immunoaffinity columns. Water Res 42:587–599

Aráoz R, Nghiêm H-O, Rippka R, Palibroda N, Tandeau de Marsac N, Herdman M (2005) Neurotoxins in axenic oscillatorian cyanobacteria: co-existence of anatoxin-a and homoanatoxin-a determined by ligand binding assay and GC/MS. Microbiology 151:1263–1273

Aráoz R, Herdman M, Rippka R, Ledreux A, Molgó J, Chanqeux J-P, Tandeau de Marsac N, Nghiêm H-O (2008) A non-readioactive ligand-binding assay for detection of cyanobacterial anatoxins using Torpedo electrocyte membranes. Toxicon 52:163–174

Arment AR, Carmichael WW (1996) Evidence that microcystin is a thio-template product. J Phycol 32:591–597

Ballot A, Pflugmacher S, Wiegand C, Kotut K, Krienitz L (2003) Cyanobacterial toxins in Lake Baringo, Kenya. Limnologica 33:2–9

Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf JS, Codd GA, Pflugmacher S (2004) Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya-Lakes Bogoria, Nakuru and Elmenteita. J Plankton Res 26:925–935

Banack SA, Johnson HE, Cheng R, Cox PA (2007) Production of the neurotoxin BMAA by a marine cyanobacterium. Mar Drugs 5:180–196

Banker R, Carmeli S, Werman M, Teltsch B, Porat R, Sukenik A (2001) Uracil moiety is required for toxicity of the cyanobacterial hepatotoxin cylindrospermopsin. J Toxicol Environ Health PtA 62:281–288

Beattie KA, Ressler J, Wiegand C, Krause E, Codd GA, Steinberg CEW, Pflugmacher S (2003) Comparative effects and metabolism of two microcystins and nodularin in the brine shrimp *Artemia salina*. Aquat Toxicol 62:219–226

Berry JP, Gantar M, Perez MH, Berry G, Noriega FG (2008) Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. Mar Drugs 6:117–146

Best JH, Pflugmacher S, Wiegand C, Eddy FB, Metcalf JS, Codd GA (2002) Effects of enteric bacterial and cyanobacterial lipopolysaccharides, and of microcystin-LR, on glutathione S-transferase activities in zebra fish (*Danio rerio*). Aquat Toxicol 60:223–231

Bláhová L, Oravec M, Maršalek B, Šejnohová L, Šimek Z, Bláha L (2009) The first occurrence of the cyanobacterial alkaloid toxin cylindrospermopsin in the Czech Republic as determined by immunochemical and LC/MS methods. Toxicon 53:519–524

Blom JF, Baumann HI, Codd GA, Jüttner F (2006) Sensitivity and adaptation of aquatic organisms to oscillapeptin J and [D-Asp^3,(E)-Dhb^7]microcystin-RR. Arch Hydrobiol 167:547–559

Bourne DG, Jones GJ, Blakeley RL, Jones A, Negri AP, Riddles P (1996) Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin-LR. Appl Environ Microbiol 62:4086–4094

Bowling JJ, Kochanowska AJ, Kasanali N, Hamann MT (2007) Nature's bounty-drug discovery from the sea. Expert Opin Drug Discov 2:1505–1522

Bownik A (2010) Harmful algae: effects of alkaloid cyanotoxins on animal and human health. Toxin Reviews 29:99–114

Braun A, Pfeiffer T (2002) Cyanobacterial blooms as the cause of a Pleistocene large mammal assemblage. Paleobiology 28:139–154

Bricelj VM, Connell L, Konoki K, MacQuarrie SP, Scheuer T, Catterall WA, Trainer VL (2005) Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. Nature 434:763–767

Brooks WP, Codd GA (1987) Distribution of *Microcystis aeruginosa* peptide toxin and interactions with hepatic microsomes in mice. Pharmacol Toxicol 60:187–191

Bruno M, Fiori M, Mattei D, Melchiorre S, Messineo V, Volpi F, Bogialli S, Nazzari M (2006) ELISA and LC-MS/MS methods for determining cyanobacterial toxins in blue-green algae food supplements. Nat Prod Res 20:827–834

Caller TA, Doolin JW, Haney JF, Murby AJ, West KG, Farrar HE, Ball A, Harris BT, Stommel EW (2009) A cluster of amyotrophic lateral sclerosis in New Hampshire: a possible role for toxic cyanobacteria blooms. Amyotroph Lateral Scler 10(S2):101–108

Carey CC, Haney JF, Cottingham KL (2007) First report of microcystin-LR in the cyanobacterium *Gloeotrichia echinulata*. Environ Toxicol 22:337–339

Carmichael WW (2008) A world overview – one hundred twenty seven years of research on toxic cyanobacteria – where do we go from here? Adv Exp Med Biol 619:104–125

Carmichael WW, Biggs DF, Gorham PR (1975) Toxicology and pharmacological action of *Anabaena flos-aquae* toxin. Science 187:542–544

Carmichael WW, Biggs DF, Peterson MA (1979) Pharmacology of anatoxin-a, produced by the freshwater cyanophyte *Anabaena flos-aquae* NRC-44-1. Toxicon 17:229–236

Carmichael WW, Beasley V, Bunner DL, Eloff JN, Falconer I, Gorham P, Harada K-I, Krishnamurthy T, Yu M-J, Moore RE, Rinehart K, Runnegar M, Skulberg OM, Watanabe M (1988) Naming of cyclic heptapeptide toxins of cyanobacteria (blue-green algae). Toxicon 26:971–973

Carmichael WW, Azevedo SMFO, An JS, Molica PJR, Jochimsen EM, Lau S, Rinehart KL, Shaw GR, Eaglesham GK (2001) Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. Environ Health Perspect 109:663–668

Chen H, Burke JM, Mosindy T, Fedorak PM, Prepas EE (2009) Cyanobacteria and microcystin-LR in a complex lake system representing a range in trophic status: Lake of the Woods, Ontario, Canada. J Plankton Res 31:993–1008

Cheng YS, Zhou Y, Irvin CM, Kirkpatrick B, Backer LC (2007) Characterization of aerosols containing microcystin. Mar Drugs 5:136–150

Childress B, Nagy S, Hughes B (2008) International single species action plan for the conservation of the Lesser Flamingo (*Phoeniconaias minor*). CMS Technical Series No. 18, AEWA Technical Series No. 34. Bonn, Germany

Christiansen G, Fastner J, Erhard M, Börner T, Dittmann E (2003) Microcystin biosynthesis in *Planktothrix*: genes, evolution and manipulation. J Bacteriol 185:564–572

Chu FS, Huang X, Wei RD, Carmichael WW (1989) Production and characterization of antibodies against microcystins. Appl Environ Microbiol 55:1928–1933

Chu FS, Huang X, Hall S (1992) Production and characterisation of antibodies against neosaxitoxin. J AOAC Int 75:341–345

Cianca RCC, Pallares MA, Barbosa RD, Adan LV, Martins JML, Gago-Martińez A (2007) Application of precolumn oxidation HPLC method with fluorescence detection to evaluate saxitoxin levels in discrete brain regions of rats. Toxicon 49:89–99

Codd GA, Bell SG (1996) The occurrence and fate of blue-green algal toxins in freshwaters. R&D Report 29, National Rivers Authority. HMSO, London, 32 pp

Codd GA, Edwards C, Beattie KA, Barr WM, Gunn GJ (1992) Fatal attraction to cyanobacteria? Nature 359:110–111

Codd GA, Steffensen DA, Burch MD, Baker PD (1994) Toxic blooms of cyanobacteria (blue-green algae) in Lake Alexandrina, South Australia – learning from history. Aust J Mar Freshw Res 45:731–736

Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS (1999a) Cyanobacterial toxins, exposure routes and human health. Eur J Phycol 34:405–415

Codd GA, Metcalf JS, Beattie KA (1999b) Retention of *Microcystis aeruginosa* and microcystin by salad lettuce (*Lactuca sativa*) after spray irrigation with water containing cyanobacteria. Toxicon 37:1181–1185

Codd GA, Metcalf JS, Ward CJ, Beattie KA, Bell SG, Kaya K, Poon GK (2001) Analysis of cyanobacterial toxins by physicochemical and biochemical methods. J AOAC Int 84:1626–1635

Codd GA, Metcalf JS, Morrison LF, Krienitz L, Ballot A, Pflugmacher S, Wiegand C, Kotut K (2003) Susceptibility of flamingos to cyanobacterial toxins via feeding. Vet Rec 152:722–723

Codd GA, Morrison LF, Metcalf JS (2005a) Cyanobacterial toxins: risk management for health protection. Toxicol Appl Pharmacol 203:264–272

Codd GA, Lindsay J, Young FM, Morrison LF, Metcalf JS (2005b) Harmful cyanobacteria: from mass mortalities to management measures. In: Huisman J, Matthijs HCP, Visser PM (eds) Harmful cyanobacteria, Aquatic ecology series. Springer, Dordrecht, pp 1–24, 242 pp

Codd GA, Azevedo SMFO, Bagchi SN, Burch MD, Carmichael WW, Harding WR, Kaya K, Utkilen HC (2005c) CYANONET: a global network for cyanobacterial bloom and toxin risk management. Initial situation assessment and recommendations. IHP-VI Technical Document in Hydrology No. 76. UNESCO, Paris, 141 pp

Colwell RR (1996) Global climate and infectious disease: the cholera paradigm. Science 274:2025–2031

Cox PA, Banack SA, Murch SJ (2003) Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. PNAS 100:13380–13383

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B (2005) Diverse taxa of cyanobacteria produce β-N-methylamino-L-alanine, a neurotoxic amino acid. PNAS 102:5074–5078

Cox PA, Richer R, Metcalf JS, Banack SA, Codd GA, Bradley WG (2009) Cyanobacteria and BMAA exposure from desert dust: a possible link to sporadic ALS among Gulf War veterans. Amyotroph Lateral Scler 10(S2):109–117

Craighead D, Metcalf JS, Banack SA, Amgalan L, Reynolds HV, Batmunkh M (2009) Presence of the neurotoxic amino acids β-N-methylamino-L-alanine (BMAA) and 2,4-diamino-butyric acid (DAB) in shallow springs from the Gobi Desert. Amyotroph Lateral Scler 10(S2):96–100

Dahms H-U, Ying X, Pfeiffer C (2006) Antifouling potential of cyanobacteria: a mini-review. Biofouling 22:317–327

Davis TW, Berry DL, Boyer GL, Gobler CJ (2009) The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae 8:715–725

Dell'Aversano C, Eaglesham GK, Quilliam MA (2004) Analysis of cyanobacterial toxins by hydrophilic interaction liquid chromatography-mass spectrometry. J Chromatogr A 1028:155–164

Depledge MH (2009) Novel approaches and technologies in pollution assessment and monitoring: a UK perspective. Ocean Coast Manag 52:336–341

Devic E, Li D, Dauta A, Henriksen P, Codd GA, Marty J-L, Fournier D (2002) Detection of anatoxin-a(S) in environmental samples of cyanobacteria by using a biosensor with engineered acetylcholinesterases. Appl Environ Microbiol 68:4102–4106

Ditmann E, Börner T (2005) Genetic contributions to the risk assessment of microcystin in the environment. Toxicol Appl Pharmacol 203:192–200

Dittmann E, Neilan BA, Erhard M, von Döhren H, Börner T (1997) Insertional mutagenesis of a peptide synthetase gene that is responsible for hepatotoxin production in the cyanobacterium *Microcystis aeruginosa* PCC7806. Mol Microbiol 26:779–787

Dörr FA, Rodríguez V, Molica R, Henriksen P, Krock B, Pinto E (2010) Methods for detection of anatoxin-a(S) by liquid chromatography coupled to electrospray ionization-tandem mass spectrometry. Toxicon 55:92–99

Dow CS, Swoboda UK (2000) Cyanotoxins. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 613–632, 229 pp

Drews G, Weckesser J (1982) Function, structure and composition of cell walls and external layers. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria. Blackwell Scientific Publications Ltd., Oxford, pp 333–357, 688 pp

Drobniewska A, Tarczyńska M, Mankiewicz J, Jurczak T, Zalewski M (2004) Increase of crustacean sensitivity to purified hepatotoxic cyanobacterial extracts by manipulation of experimental conditions. Environ Toxicol 19:416–420

Edelman MJ, Gandara DR, Hausner P, Israel V, Thornton D, DeSanto J, Doyle LA (2003) Phase 2 study of cryptophycin 52 (LY355703) in patients previously treated with platinum based chemotherapy for advanced non-small cell lung cancer. Lung Cancer 39:197–199

Edwards C, Beattie KA, Scrimgeour CM, Codd GA (1992) Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. Toxicon 30:1165–1175

Edwards C, Lawton LA, Beattie KA, Codd GA, Pleasance S, Dear GJ (1993) Analysis of microcystins from cyanobacteria by liquid chromatography with mass spectrometry using atmospheric-pressure ionization. Rapid Commun Mass Spectrom 7:714–721

Erhard M, Von Döhren H, Jungblut P (1997) Rapid typing and elucidation of new secondary metabolites of intact cyanobacteria using MALDI-TOF mass spectrometry. Nat Biotechnol 15:906–909

Erpenbeck D, van Soest RWM (2007) Status and potential of sponge chemosystematics. Mar Biotechnol 9:2–19

Erridge C, Bennett-Guerrero R, Poxton IR (2002) Structure and function of lipopolysaccharides. Microbes Infect 4:837–851

Esterhuizen M, Downing TG (2008) β-N-methylamino-L-alanine (BMAA) in novel South African cyanobacterial isolates. Ecotoxicol Environ Saf 71:309–313

Etheridge SM (2010) Paralytic shellfish poisoning: seafood safety and human health perspectives. Toxicon 56:108–122

Faassen EJ, Gillissen F, Zweers HAJ, Lürling M (2009) Determination of the neurotoxins BMAA (β-N-methylamino-L-alanine) and DAB (α, γ-diaminobutyric acid) by LC-MSMS in Dutch urban waters with cyanobacterial blooms. Amyotroph Lateral Scler 10(S2):79–84

Falconer IR (2004) Cyanobacterial toxins of drinking water supplies: cylindrospermopsins and microcystins. CRC Press, Boca Raton, 296 pp

Falconer IR, Humpage AR (2005) Health risk assessment of cyanobacterial (blue-green algal) toxins in drinking water. Int J Environ Res Public Health 2:43–50

Falconer IR, Buckley T, Runnegar MT (1986) Biological half-life, organ distribution and excretion of ^{125}I-labelled toxic peptides from the blue-green alga *Microcystis aeruginosa*. Aust J Biol Sci 39:17–21

Falconer IR, Choice A, Hosja W (1992) Toxicity of edible mussels (*Mytilus edulis*) growing naturally in an estuary during a water bloom of the blue-green alga *Nodularia spumigena*. Environ Toxicol Water Qual 7:119–123

Falconer IR, Burch MD, Steffensen DA, Choice M, Coverdale OR (1994) Toxicity of the blue-green alga (cyanobacterium) *Microcystis aeruginosa* in drinking water to growing pigs, as an animal model for human injury and risk assessment. Environ Toxicol Water Qual 9:131–139

Fastner J, Flieger I, Neumann U (1998) Optimised extraction of microcystins from field samples- a comparison of different solvents and procedures. Water Res 32:3177–3181

Feurstein D, Holst K, Fischer A, Dietrich DR (2009) Oatp-associated uptake and toxicity of microcystins in primary murine whole brain cells. Toxicol Appl Pharmacol 234:247–255

Fischer WJ, Garthwaite I, Miles CO, Ross KM, Aggen JB, Chamberlin RA, Towers NR, Dietrich DR (2001) Congener-independent immunoassay for microcystins and nodularins. Environ Sci Technol 35:4849–4856

Fitzgeorge RB, Clark SA, Keevil CW (1994) Routes of intoxication. In: Codd GA, Jeffries TM, Keevil CW, Potter E (eds) Detection methods for cyanobacterial toxins (proceedings of the first international symposium on detection methods for cyanobacterial (blue-green algal) toxins). The Royal Society of Chemistry, Cambridge, pp 69–74

Francis G (1878) Poisonous Australian lake. Nature 18:1–12

Froscio SM, Humpage AR, Burcham PC, Falconer IR (2001) Cell-free protein synthesis inhibition assay for the cyanobacterial toxin cylindrospermopsin. Environ Toxicol 16:408–412

Funari E, Testai E (2008) Human health risk assessment related to cyanotoxins exposure. Crit Rev Toxicol 38:97–125

Furey A, Crowley J, Lehane M, James KJ (2003) Liquid chromatography with electrospray ion-trap mass spectrometry for the determination of anatoxins in cyanobacteria and drinking water. Rapid Commun Mass Spectrom 17:583–588

Gácsi M, Antal O, Vasas G, Máthé C, Borbély G, Saker ML, Gyori J, Farkas A, Vehovszky A, Bánfalvi G (2009) Comparative study of cyanotoxins affecting cytoskeletal and chromatin structures in CHO-K1 cells. Toxicol In Vitro 23:710–718

Gademann K, Kobylinska J (2009) Antimalarial natural products of marine and freshwater origin. Chem Rec 9:187–198

Gademann K, Portmann C (2008) Secondary metabolites from cyanobacteria: complex structures and powerful bioactivities. Curr Org Chem 12:326–341

Gantar M, Svirčev Z (2008) Microalgae and cyanobacteria: food for thought. J Phycol 44:260–268

Gee CE, Mansuy IM (2005) Protein phosphatases and their potential implications in neuroprotective processes. Cell Mol Life Sci 62:1120–1130

Gerwick WH, Tong Tan L, Sitachitta N (2001) Nitrogen-containing metabolites from marine cyanobacteria. Alkaloids Chem Biol 57:75–184

Giannuzzi L, Sedan D, Echenique R, Androlio D (2011) An acute case of intoxication with cyanobacteria and cyanotoxins in recreational water in Salto Grande Dam, Argentina. Mar Drugs 9:2164–2175

Harada K-I, Ogawa K, Matsuura K, Nagai H, Murata H, Suzuki M, Itezono Y, Nakayama N, Shirai M, Nakano M (1991) Isolation of two toxic heptapeptide microcystins from an axenic strain of *Microcystis aeruginosa*, K-139. Toxicon 29:479–489

Harada K, Fujii K, Shimada T, Suzuki M, Sano H, Adachi K, Carmichael WW (1995) Two cyclic peptides, anabaenopeptins, a third group of bioactive compounds from the cyanobacterium *Anabaena flos-aquae* NRC 525-17. Tetrahedron Lett 36:1511–1514

Harrigan GG, Goetz G (2002) Symbiotic and dietary marine microalgae as a source of bioactive molecules- experience from natural products research. J Appl Phycol 14:103–108

Hastie CJ, Borthwick EB, Morrison LF, Codd GA, Cohen PTW (2005) Inhibition of several protein phosphatases by a non-covalently interacting microcystin and a novel cyanobacterial peptide, nostocyclin. Biochim Biophys Acta 1726:187–193

Hawkins PR, Runnegar MTC, Jackson ARB, Falconer IR (1985) Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. Appl Environ Microbiol 50:1292–1295

Hilborn ED, Carmichael WW, Soares RM, Yuan M, Servaites JC, Barton HA, Azevedo SMFO (2007) Serologic evaluation of human microcystin exposure. Environ Toxicol 22:459–463

Ho L, Newcombe G (2007) Evaluating the adsorption of microcystin toxins using granular activated carbon (GAC). J Water Supply Res Technol AQUA 56:281–291

Ho L, Slyman N, Kaeding U, Newcombe G (2008) Optimizing PAC and chlorination practices for cylindrospermopsin removal. J AWWA 100:88–96

Ho L, Sawade E, Newcombe G (2012) Biochemical treatment options for cyanobacterial metabolite removal. Water Research 46:1536–1548

Hudnell HK (ed) (2008) Cyanobacterial harmful algal blooms: state of the science and research needs. Adv Exp Med 619:950

Hughes S (1996) Lidocaine- possible neurotoxicity. Int J Obstet Anesth 5:1–2

Humpage AR (2008) Toxin types, toxicokinetics and toxicodynamics. Adv Exp Med Biol 619:383–415

Humpage AR, Falconer IR (2003) Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. Environ Toxicol 18:94–103

Imanishi S, Kato H, Mizuno M, Tsuji K, Harada K-I (2005) Bacterial degradation of microcystins and nodularin. Chem Res Toxicol 18:591–598

Ince PG, Codd GA (2005) Return of the cycad hypothesis- does the amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC) of Guam have implications for global health? Neuropathol Appl Neurobiol 31:345–353

Ishii S, Sadowsky MJ (2008) *Escherichia coli* in the environment: Implications for water quality and human health. Microbes Environ 23:101–108

Islam MS, Hasan MK, Miah MA, Yunus M, Zaman K, Albert MJ (1994) Isolation of *Vibrio cholerae* O139 synonym Bengal from the aquatic environment in Bangladesh: implications for disease transmission. Appl Environ Microbiol 60:1684–1686

Islam MS, Jahid MIK, Rahman MM, Rahman MZ, Shafiqu M, Islam MSK, Sack DA, Schoolnik GK (2007) Biofilm acts as a microenvironment for plankton-associated *Vibrio cholerae* in the aquatic environment of Bangladesh. Microbiol Immunol 51:369–379

Ito E, Nagai H (1998) Morphological observations of diarrhea in mice caused by aplysiatoxin, the causative agent of the red alga *Gracilaria coronopifolia* poisoning in Hawaii. Toxicon 36:1913–1920

Ito E, Nagai H (2000) Bleeding from the small intestine caused by aplysiatoxin, the causative agent of the red alga *Gracilaria coronopifolia* poisoning. Toxicon 38:123–132

Jaiswal P, Singh PK, Prasanna R (2008) Cyanobacterial bioactive molecules- an overview of their toxic properties. Can J Microbiol 54:701–717

James KJ, Furey A, Sherlock IR, Stack MA, Twohig M, Caudwell FB, Skulberg OM (1998) Sensitive determination of anatoxin-a, homoanatoxin-a and their degradation products by liquid chromatography with fluorimetric detection. J Chromatogr A 798:137–145

Jochimsen EM, Carmichael WW, An J, Cardo DM, Cookson ST, Holmes CEM, De Antunes MBC, De Melo Filho DA, Lyra TM, Barreto VST, Azevedo SMFO, Javis WR (1998) Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. N Engl J Med 338:873–878

Jonasson S, Eriksson J, Berntzon L, Spáčil Z, Ilag LL, Ronnevi L-O, Rasmussen U, Bergman B (2010) Transfer of a cyanobacterial neurotoxin within a temperate aquatic ecosystem suggests pathways for human exposure. PNAS 107:9252–9257

Kankaanpää HT, Sjövall O, Huttunen M, Olin M, Karlsson K, Hyvärin K, Sneitz L, Härkönen J, Sipiä VO, Meriluoto JAO (2009) Production and sedimentation of peptide toxins nodularin-R and microcystin-LR in the northern Baltic Sea. Environ Pollut 157:1301–1309

Kato H, Tsuji K, Harada KI (2009) Microbial degradation of cyclic peptides produced by cyanobacteria. J Antibiot 62:181–190

Kaya K, Mahakhant A, Keovara L, Sano T, Kubo T, Takaqi H (2002) Spiroidesin, a novel lipopeptide from the cyanobacterium *Anabaena spiroides* that inhibits cell growth of the cyanobacterium *Microcystis aeruginosa*. J Nat Prod 65:920–921

Kehr J-C, Picchi DG, Dittman E (2011) Natural product biosynthesis in cyanobacteria: a treasure trove of unique enzymes. Beilsten J Org Chem 7:1622–1635

Kellmann R, Michali TK, Neilan BA (2008) Identification of a saxitoxin biosynthesis gene with a history of frequent horizontal gene transfers. J Mol Evol 67:526–538

Kfir R, Johannsen E, Botes DP (1986) Monoclonal antibody specific to cyanoginosin-LA: preparation and characterization. Toxicon 24:543–552

Khreich N, Lamourette P, Renard P-Y, Clavé G, Fenaille F, Créminon C, Volland H (2009) A highly sensitive competitive immunoassay of broad specificity quantifying microcystins and nodularins in water samples. Toxicon 53:551–559

Kokociński M, Dziga D, Spoof L, Stefaniak K, Jurczak T, Mankiewicz-Boczek J, Meriluoto J (2009) First report of the cyanobacterial toxin cylindrospermopsin in the shallow, eutrophic lakes of western Poland. Chemosphere 74:669–675

Kondo F, Harada K-I, Ueno Y (2005) Method for quantifying microcystins in fish using immunoaffinity purification. J Liq Chromatogr Relat Technol 28:3025–3033

Kotut K, Ballot A, Wiegand C, Krienitz L (2010) Toxic cyanobacteria at Nakuru sewage oxidation ponds- a potential threat to wildlife. Limnologica 40:47–53

Krienitz L, Ballot A, Kotut K, Wiegand C, Pütz S, Metcalf JS, Codd GA, Pflugmacher S (2003) Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. FEMS Microbiol Ecol 43:141–148

Krienitz L, Ballot A, Casper P, Codd GA, Kotut K, Metcalf JS, Morrison LF, Pflugmacher S, Wiegand C (2005) Contribution of toxic cyanobacteria to massive deaths of Lesser Flamingos at saline-alkaline lakes of Kenya. Verh Int Verein Limnol 29:783–786

Krüger T, Mönch B, Oppenhäuser S, Luckas B (2010) LC-MS/MS determination of the isomeric neurotoxins BMAA (β-N-methylamino-L-alanine) and DAB (2,4-diaminobutyric acid) in cyanobacteria and seeds of *Cycas revoluta* and *Lathyrus latifolius*. Toxicon 55:547–557

Kubo T, Sano T, Hosoya K, Tanaka N, Kaya K (2005) A new simple and effective fractionation method for cylindrospermopsin analyses. Toxicon 46:104–107

Kubo T, Kato N, Hosoya K, Kaya K (2008) Effective determination method for a cyanobacterial neurotoxin, β-N-methylamino-L-alanine. Toxicon 51:1264–1268

Lawrence JF, Maher M, Watson-Wright W (1994) Effect of cooking on the concentration of toxins associated with paralytic shellfish poison in lobster hepatopancreas. Toxicon 32:57–64

Lawton LA, Edwards C, Codd GA (1994) Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. Analyst 119:1525–1530

Lawton LA, Chambers H, Edwards C, Nwaopara AA, Healy M (2010) Rapid detection of microcystins in cells and water. Toxicon 55:973–978

Leflaive J, Ten-Hage L (2007) Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. Freshw Biol 52:199–214

Lemmens-Gruber R, Kamyar MR, Dornetshuber R (2009) Cyclodepsipeptides- potential drugs and lead compounds in the drug development process. Curr Med Chem 16:1122–1137

Li H, Xie P, Li G, Hao L, Xiong Q (2009) In vivo study on the effects of microcystin extracts on the expression profiles of proto-oncogenes (c-fos, c-jun and c-myc) in liver, kidney and testis of male Wistar rats injected i.v. with toxins. Toxicon 53:169–175

Lindsay J, Metcalf JS, Codd GA (2006) Protection against the toxicity of microcystin-LR and cylindrospermopsin in *Artemia salina* and *Daphnia* spp. by pre-treatment with cyanobacterial lipopolysaccharide (LPS). Toxicon 48:995–1001

Lindsay J, Metcalf JS, Codd GA (2009) Comparison of four methods for the extraction of lipopolysaccharide from cyanobacteria. Toxicol Environ Chem 91:1253–1262

Lippy EC, Erb J (1976) Gastrointestinal illness at Sewickley, PA. J AWWA 68:606–610

Liu Y, Xie P, Xiao-Ping W (2009) Grazing on toxic and non-toxic *Microcystis aeruginosa* PCC7820 by *Unio boudlasiae* and *Corbicula fluminea*. Limnology 10:1–5

Llewellyn LE (2006) Saxitoxin, a toxic marine natural product that targets a multitude of receptors. Nat Prod Rep 23:200–222

Llewellyn LE (2009) Sodium channel inhibiting marine toxins. Prog Mol Subcell Biol 46:67–97

Llewellyn L, Negri A, Robertson A (2006) Paralytical shellfish toxins in tropical oceans. Toxicol Rev 25:159–196

MacKintosh C, Beattie KA, Klumpp S, Cohen P, Codd GA (1990) Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett 264:187–192

Mahar J, Lukacs GL, Li Y, Hall S, Moczydlowski E (1991) Pharmacological and biochemical properties of saxiphilin, a soluble saxitoxin-binding protein from the bullfrog (*Rana catesbeiana*). Toxicon 29:53–71

Mahmood NA, Carmichael WW (1986) The pharmacology of anatoxin-a(S), a neurotoxin produced by the freshwater cyanobacterium *Anabaena flos-aquae* NRC 525-17. Toxicon 24:425–434

Mahmood NA, Carmichael WW (1987) Paralytic shellfish poisons produced by the freshwater cyanobacterium *Aphanizomenon flos-aquae* NH-5. Toxicon 24:175–186

Marahiel MA, Stachelhaus T, Mootz HD (1997) Modular peptide synthetases involved in nonribosomal peptide synthesis. Chem Rev 97:2651–2673

Martin C, Codd GA, Siegelman HW, Weckesser J (1989) Lipopolysaccharides and polysaccharides of the cell envelope of toxic *Microcystis aeruginosa* strains. Arch Microbiol 152:90–94

Matsunaga S, Moore RE, Niemczura WP, Carmichael WW (1989) Anatoxin-a(S), a potent anticholinesterase from *Anabaena flos-aquae*. J Am Chem Soc 111:8021–8023

Mazur-Marzec H, Plínski M (2009) Do toxic cyanobacteria blooms pose a threat to the Baltic ecosystem? Oceanologia 51:239–319

Mazur-Marzec H, Spoof L, Kobos J, Plínski M, Meriluoto J (2008) Cyanobacterial hepatotoxins, microcystins and nodularins, in fresh and brackish waters of the Pomeranian Province, northern Poland. Oceanol Hydrol Stud 37:3–21

McElhiney J, Lawton LA (2005) Detection of the cyanobacterial hepatotoxins microcystins. Toxicol Appl Pharmacol 203:219–230

Meriluoto J, Codd GA (2005) Toxic cyanobacterial monitoring and cyanotoxin analysis. Åbo Akademi Press, Åbo, 149 pp

Metcalf JS, Codd GA (2000) Microwave oven and boiling waterbath extraction of hepatotoxins from cyanobacterial cells. FEMS Microbiol Lett 184:241–246

Metcalf JS, Codd GA (2003) Analysis of cyanobacterial toxins by immunological methods. Chem Res Toxicol 16:103–112

Metcalf JS, Codd GA (2004) Cyanobacterial toxins in the water environment: a review of current knowledge. Foundation for Water Research, Marlow, 36 pp

Metcalf JS, Codd GA (2009) The status and potential of cyanobacteria and their toxins as agents of bioterrorism. In: Gault PM, Marler HJ (eds) Handbook on cyanobacteria: biochemistry, biotechnology and applications. Nova Publishers, New York

Metcalf JS, Beattie KA, Pflugmacher S, Codd GA (2000a) Immunocross reactivity and toxicity assessment of conjugation products of the cyanobacterial toxin, microcystin-LR. FEMS Microbiol Lett 189:155–158

Metcalf JS, Bell SG, Codd GA (2000b) Production of novel polyclonal antibodies against the cyanobacterial toxin microcystin-LR and their application for the detection and quantification of microcystins and nodularin. Water Res 34:2761–2769

Metcalf JS, Bell SG, Codd GA (2001) Colorimetric immuno-protein phosphatase inhibition assay for specific detection of microcystins and nodularins of cyanobacteria. Appl Environ Microbiol 67:904–909

Metcalf JS, Beattie KA, Saker ML, Codd GA (2002a) Effects of organic solvents on the high performance liquid chromatographic analysis of the cyanobacterial toxin cylindrospermopsin and its recovery from environmental eutrophic water by solid phase extraction. FEMS Microbiol Lett 216:159–164

Metcalf JS, Lindsay J, Beattie KA, Birmingham S, Saker ML, Törökné AK, Codd GA (2002b) Toxicity of cylindrospermopsin to the brine shrimp *Artemia salina*: comparisons with protein synthesis inhibitors and microcystins. Toxicon 40:1115–1120

Metcalf JS, Barakate A, Codd GA (2004) Inhibition of plant protein synthesis by the cyanobacterial hepatotoxin cylindrospermopsin. FEMS Microbiol Lett 235:125–129

Metcalf JS, Meriluoto JAO, Codd GA (2006a) Legal and security requirements for the air transportation of cyanotoxins and toxigenic cyanobacterial cells for legitimate research and analytical purposes. Toxicol Lett 163:85–90

Metcalf JS, Morrison LF, Krienitz L, Ballot A, Krause E, Kotut K, Pütz S, Wiegand C, Pflugmacher S, Codd GA (2006b) Analysis of the cyanotoxins anatoxin-a and microcystins in Lesser Flamingo feathers. Toxicol Environ Chem 88:159–167

Metcalf JS, Banack SA, Lindsay J, Morrison LF, Cox PA, Codd GA (2008) Co-occurrence of β-N-methylamino-L-alanine, a neurotoxic amino acid with other cyanobacterial toxins in British waterbodies, 1990–2004. Environ Microbiol 10:702–708

Metcalf JS, Reilly M, Young FM, Codd GA (2009) Localization of microcystin synthetase genes in colonies of the cyanobacterium *Microcystis* using fluorescence *in situ* hybridization. J Phycol 45:1400–1404

Metcalf JS, Richer R, Cox PA, Codd GA (2012) Cyanotoxins in desert environments may present a risk to human health. Sci Total Env 421–422, 118–123

Mez K, Beattie KA, Codd GA, Hanselmann K, Hauser B, Naegeli H, Preisig HR (1997) Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. Eur J Phycol 32:111–117

Mikhailov A, Härmälä-Braskén A-S, Polosukhina E, Hanski A, Wahlsten M, Sivonen K, Eriksson JE (2001) Production and specificity of monoclonal antibodies against nodularin conjugated through N-methyldehydrobutyrine. Toxicon 39:1453–1459

Moffitt MC, Neilan BA (2004) Characterization of the nodularin synthetase gene cluster and proposed theory of the evolution of cyanobacterial toxins. Appl Environ Microbiol 70:6353–6362

Mohamed ZA, El-Sharouny HM, Ali WSF (2006) Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigation canals, Egypt. Toxicon 47:584–590

Molica RJR, Oliveira EJA, Carvalho PVVC, Costa ANSF, Cunha MCC, Melo GL, Azevedo SMFO (2005) Occurrence of saxitoxins and an anatoxin-a(S)-like anticholinesterase in a Brazilian drinking water supply. Harmful Algae 4:743–753

Montine TJ, Li K, Perl DP, Galasko D (2005) Lack of β-methylamino-L-alanine in brain from controls, AD, or Chamorros with PDC. Neurology 65:768–769

Moore RE, Ohtani I, Moore BS, de Koning CB, Yoshida WY, Runnegar MTC, Carmichael WW (1993) Cyanobacterial toxins. Gazz Chim Ital 123:329–336

Moorhead GBG, Trinkle-Mulcahy L, Ulke-Lemée A (2007) Emerging roles of nuclear protein phosphatases. Nat Rev Mol Cell Biol 8:234–244

Nishiwaki-Matsushima R, Ohta T, Nishiwaki S, Suganuma M, Kohyama K, Ishikawa T, Carmichael W, Fujiki H (1992) Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. J Cancer Res Clin Oncol 118:420–424

Nishizawa T, Asayama M, Fujii K, Harada KI, Shirai M (1999) Genetic analysis of the peptide synthetase genes for a cyclic heptapeptide microcystin in *Microcystis* spp. J Biochem 126:520–529

Nishizawa T, Ueda A, Asayama M, Fujii K, Harada KI, Ochi K, Shirai M (2000) Polyketide synthase gene coupled to the peptide synthetase module involved in the biosynthesis of the cyclic heptapeptide microcystin. J Biochem 127:779–789

Norris RL, Eaglesham GK, Pierens G, Shaw GR, Smith MJ, Chiswell RK, Seawright AA, Moore MR (1999) Deoxyclindrospermopsin, an analog of cylindrospermopsin from *Cylindrospermopsis raciborskii*. Environ Toxicol 14:163–165

Norris RLG, Eaglesham GK, Shaw GR, Senogles P, Chiswell RK, Smith MJ, Davis BC, Seawright AA, Moore MR (2001) Extraction and purification of the zwitterions cylindrospermopsin and deoxycylindrospermopsin from *Cylindrospermopsis raciborskii*. Environ Toxicol 16:391–396

Norris RLG, Seawright AA, Shaw GR, Senogles P, Eaglesham GK, Smith MJ, Chiswell RK, Moore MR (2002) Hepatic xenobiotic metabolism of cylindrospermopsin in vivo in the mouse. Toxicon 40:471–476

Oberholster PJ, Myburgh JG, Govender D, Bengis R, Botha A-R (2009a) Identification of toxigenic *Microcystis* strains after incidents of wild animal mortalities in the Kruger National Park, South Africa. Ecotoxicol Environ Saf 72:1177–1182

Oberholster PJ, Mthethwa BLS, Botha AM (2009b) Development of a rapid and sensitive battery of bioassays for risk assessment of cyanobacterial microcystin-LR in drinking water or rural treatment plants, South Africa. Afr J Biotechnol 8:4562–4571

Ortelli D, Edder P, Cognard E, Jan P (2008) Fast screening and quantitation of microcystins in microalgae dietary supplement products and water by liquid chromatography coupled to time of flight mass spectrometry. Anal Chim Acta 617:230–237

Osborne NJT, Webb PM, Shaw GR (2001) The toxins of *Lyngbya majuscula* and ecological health effects. Environ Int 27:381–392

Pablo J, Banack SA, Cox PA, Johnson TE, Papapetropoulos S, Bradley WG, Buck A, Mash DC (2009) Cyanobacterial neurotoxin BMAA in ALS and Alzheimer's disease. Acta Neurol Scand 120:216–225

Paul VJ, Ritson-Williams R (2008) Marine benthic invertebrates use multimodal cues for defense. Nat Prod Rep 25:662–695

Pouria S, De Andrade A, Barbosa J, Cavalcanti RL, Barreto VTS, Ward CJ, Preiser W, Poon GK, Neild GH, Codd GA (1998) Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. Lancet 352:21–26

Purdie EL, Young FM, Menzel D, Codd GA (2009) A method for acetonitrile-free microcystin analysis and purification by high performance liquid chromatography, using methanol as mobile phase. Toxicon 54:887–890

Rantala A, Fewer DP, Hisbergues M, Rouhiainen L, Vaitomaa J, Börner T, Sivonen K (2004) Phylogenetic evidence for the early evolution of microcystin synthesis. PNAS 101:568–573

Rapala J, Sivonen K, Luukkainen R, Niemela SI (1993) Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena* strains- a laboratory study. J Appl Phycol 5:581–591

Raziuddin S, Siegelman HW, Tornabene TG (1983) Lipopolysaccharides of the cyanobacterium *Microcystis aeruginosa*. Eur J Biochem 137:333–336

Rellán S, Osswald J, Saker M, Gago-Martinez A, Vasconcelos V (2009) First detection of anatoxin-a in human and animal dietary supplements containing cyanobacteria. Food Chem Toxicol 47:2189–2195

Rivasseau C, Racaud P, Deguin A, Hennion M-C (1999) Development of a bioanalytical phosphatase inhibition test for the monitoring of microcystins in environmental water samples. Anal Chim Acta 394:243–257

Robinson NA, Pace JG, Matson CF, Miura GA, Lawrence WB (1991) Tissue distribution, excretion and hepatic biotransformation of microcystin-LR in mice. J Pharmacol Exp Ther 256:176–182

Rodríguez E, Onstad GD, Kull TPJ, Metcalf JS, Acero JL, von Gunten U (2007) Oxidative elimination of cyanotoxins: comparisons of ozone, chlorine, chlorine dioxide and permanganate. Water Res 41:3381–3393

Rohrlack T, Christoffersen K, Hansen PE, Zhang W, Czarnecki O, Henning M, Fastner J, Erhard M, Neilan BA, Kaebernick M (2003) Isolation, characterization, and quantitative analysis of microviridin J, a new *Microcystis* metabolite toxic to *Daphnia*. J Chem Ecol 29:1757–1770

Rohrlack T, Christoffersen K, Kaebernick M, Neilan BA (2004) Cyanobacterial protease inhibitor microviridin J causes a lethal molting disruption in *Daphnia pulicaria*. Appl Environ Microbiol 70:5047–5050

Rosén J, Hellenäs K-E (2008) Determination of the neurotoxin BMAA (β-N-methylamino-L-alanine) in cycad seed and cyanobacteria by LC-MS/MS (liquid chromatography tandem mass spectrometry). Analyst 133:1785–1789

Rositano J, Newcombe G, Nicholson B, Sztajnbok P (2001) Ozonation of NOM and algal toxins in four treated waters. Water Res 35:23–32

Ruecekert A, Cary SC (2009) Use of an armoured RNA standard to measure microcystin synthetase E gene expression in toxic *Microcystis* sp. by reverse transcription QPCR. Limnol Oceanogr Method 7:509–520

Runnegar MTC, Gerdes RG, Falconer IR (1991) The uptake of the cyanobacterial hepatotoxin microcystin by isolated rat hepatocytes. Toxicon 29:43–51

Saker ML, Thomas AD, Norton JH (1999) Cattle mortality attributed to the toxic cyanobacterium *Cylindrospermopsis raciborskii* in an outback region of north Queensland. Environ Toxicol 14:179–182

Sano T, Takagi H, Morrison LF, Metcalf JS, Codd GA, Kaya K (2005) Leucine aminopeptidase M inhibitors, cyclostatins A and B, isolated from cyanobacterial water blooms in Scotland. Phytochemistry 66:543–548

Sano T, Kikuchi S, Kubo T, Takagi H, Hosoya K, Kaya K (2008) New values of molecular extinction coefficient and specific rotation for cyanobacterial toxin cylindrospermopsin. Toxicon 51:717–719

Schembri MA, Neilan BA, Saint CP (2001) Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. Environ Toxicol 16:413–421

Schripsema J, Dagnino D (2002) Complete assignment of the NMR spectra of [D-Leu^1]-microcystin-LR and analysis of its structure. Magn Reson Chem 40:614–617

Sipiä VO, Neffling M-R, Metcalf JS, Nybom SMK, Meriluoto J.A.O, Codd GA (2008) Nodularin in feathers and livers of eiders (*Somateria mollissima*) caught from the western Gulf of Finland in June-September 2005. Harmful Algae 7:99–105

Sivonen K, Jones G (1999) Cyanobacterial toxins. In: Chorus I, Bartram J (eds) Toxic cyanobacteria in water: a guide to their public health consequences. Monitoring and management. E & FN Spon, London, pp 55–124

Skulberg OM, Carmichael WW, Andersen RA, Matsunaga S, Moore RE, Skulberg R (1992) Investigations of a neurotoxic oscillatorialean strain (cyanophyceae) and its toxin. Isolation and characterization of homoanatoxin-a. Environ Toxicol Chem 11:321–329

Smith JL, Boyer GL, Zimba PV (2008) A review of cyanobacterial odorous and bioactive metabolites: impacts and management alternatives in aquaculture. Aquaculture 280:5–20

Spácil Z, Eriksson J, Jonasson S, Rasmussen U, Ilag LL, Bergman B (2010) Analytical protocol for identification of BMAA and DAB in biological specimens. Analyst 135:127–132

Spoof L, Berg KA, Rapala J, Lahti K, Lepistö L, Metcalf JS, Codd GA, Meriluoto J (2006) First observation of cylindrospermopsin in *Anabaena lapponica* isolated from the boreal environment (Finland). Environ Toxicol 21:552–560

Spoof L, Neffling M-R, Meriluoto J (2009) Separation of microcystins and nodularins by ultra performance liquid chromatography. J Chromatogr B Anal Technol Biomed Life Sci 877:3822–3830

Spoof L, Neffling M-R, Meriluoto J (2010) Fast separation of microcystins and nodularins on narrow-bore reversed-phase columns coupled to a conventional HPLC system. Toxicon 55(5):954–964

Stewart I, Webb PM, Schluter PJ, Fleming LE, Burns JW, Gantar M, Backer LC, Shaw GR (2006) Epidemiology of recreational exposure to freshwater cyanobacteria – an international prospective cohort study. BMC Public Health 6:93–104

Stommel E, Watters MR (2004) Marine neurotoxins: ingestible toxins. Curr Treat Options Neurol 6:105–114

Svircev Z, Krstic S, Miladinov-Mikov M, Baltic V, Vidovic M (2009) Freshwater cyanobacterial blooms and primary liver cancer epidemiological studies in Serbia. J Environ Sci Health C 27:36–55

Tan LK (2007) Bioactive natural products from marine cyanobacteria for drug discovery. Phytochemistry 68:954–979

Tarczynska M, Nalecz-Jawecki G, Romanowska-Duda Z, Sawicki J, Beattie KA, Codd GA, Zalewski M (2001) Tests for the toxicity assessment of cyanobacterial bloom samples. Environ Toxicol 16:383–390

Teixeira MG, Costa MC, de Carvalho VL, Pereira MS, Hage E (1993) Gastroenteritis epidemic in the area of Itaparica Dam, Bahia, Brazil. Bull Pan Am Health Organ 27:244–253

Terao K, Ohmori S, Igarashi K, Ohtani I, Watanabe MF, Harada K-I, Ito E, Watanabe M (1994) Electron microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans*. Toxicon 32:833–843

Thajuddin N, Subramanian G (2005) Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci 89:47–57

Tillett D, Dittmann E, Erhard M, von Döhren H, Börner T, Neilan BA (2000) Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide-polyketide synthetase system. Chem Biol 7:753–764

Tippkötter N, Stückmann H, Kroll S, Winkelmann G, Noack U, Scheper T, Ulber R (2009) A semi-quantitative dipstick assay for microcystin. Anal Bioanal Chem 394:863–869

Törökné A, Asztalos M, Bánkiné M, Bickel H, Borbély G, Carmeli S, Codd GA, Fastner J, Huang Q, Humpage A, Metcalf JS, Rábai E, Sukenik A, Surányi G, Vasas G, Weiszfeiler V (2004) Interlaboratory comparison trial on cylindrospermopsin measurement. Anal Biochem 332:280–284

Turner PC, Gammie AJ, Hollinrake K, Codd GA (1990) Pneumonia associated with contact with cyanobacteria. BMJ 6737:1440–1441

Ueno Y, Nagata S, Tsutsumi T, Hasegawa A, Watanabe MF, Park H-D, Chen G-C, Chen G, Yu S-Z (1996) Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis 17:1317–1321

van Appeldoorn ME, van Egmond HP, Speijers GJ, Bakker GJ (2007) Toxins of cyanobacteria. Mol Nutr Food Res 51:7–60

van Wagoner RM, Drummond AK, Wright JLC (2007) Biogenetic diversity of cyanobacterial metabolites. Adv Appl Microbiol 61:89–217

Varricchio DJ (1995) Taphonomy of Jack's Birthday Site, a diverse dinosaur bonebed from the Upper Cretaceous Two Medicine Formation of Montana. Palaeogeogr Palaeoclimatol Palaeoecol 114:297–323

Vasas G, Gaspar A, Suranyi G, Batta G, Gyemant G, M-Hamvas M, Mathe C, Grigorszky I, Molnar E, Borbely G (2002) Capillary electrophoretic assay and purification of cylindrospermopsin, a cyanobacterial toxin from *Aphanizomenon ovalisporum*, by plant test (Blue-Green Sinapsis Test). Anal Biochem 302:95–103

Vásquez M, Gruttner C, Gallacher S, Moore ERB (2001) Detection and characterization of toxigenic bacteria associated with *Alexandrium catanella* and *Aulacomya ater* contaminated with PSP. J Shellfish Res 20:1245–1249

Vega A, Bell EA (1967) α-amino-β-methylaminopropionic acid, a new amino acid from seeds of *Cycas circinalis*. Phytochemistry 6:759–762

Wang J, Pang X, Ge F, Ma Z (2007) An ultra-performance liquid chromatography-tandem mass spectrometry method for determination of microcystins occurrence in surface water of Zhejiang Province, China. Toxicon 49:1120–1128

Wang Q, Xie P, Chen J, Liang G (2008) Distribution of microcystins in various organs (heart, liver, intestine, gonad, brain, kidney and lung) of Wistar rat via intravenous injection. Toxicon 52:721–727

Ward CJ, Beattie KA, Lee EYC, Codd GA (1997) Colorimetric protein phosphatase inhibition assay of laboratory strains and natural blooms of cyanobacteria: comparisons with high-performance liquid chromatographic analysis of microcystins. FEMS Microbiol Lett 153:465–473

Wase NV, Wright PC (2008) Systems biology of cyanobacterial secondary metabolite production and its role in drug discovery. Expert Opin Drug Discov 3:903–929

Watson SB (2003) Cyanobacterial and eukaryotic algal odour compounds: signals or by-products? A review of their biological activity. Phycologia 42:332–350

Welker M, von Döhren H (2006) Cyanobacterial peptides- nature's own combinatorial biosynthesis. FEMS Microbiol Rev 30:530–563

Whitton BA, Potts M (2000) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, 669 pp

Wiegand C, Pflugmacher S (2005) Ecotoxicological effects of selected cyanobacterial secondary metabolites. Toxicol Appl Pharmacol 203:201–218

Wood SA, Dietrich DR, Craig Cary S, Hamilton DP (2012) Increasing *Microcystis* cell density enhances microcystin synthesis: a mesocosm study. Inland Waters 2:17–22

Wormer L, Cirés S, Carrasco D, Quesada A (2008) Cylindrospermopsin is not degraded by co-occurring natural bacteria communities during a 40-day study. Harmful Algae 7:206–213

Wormer L, Carrasco D, Cirés S, Quesada A (2009) Advances in the solid phase extraction of the cyanobacterial toxin cylindrospermopsin. Limnol Oceanogr Method 7:568–575

Wulff J (2006) Ecological interactions of marine sponges. Can J Zool 84:146–166

Young FM, Metcalf JS, Meriluoto JAO, Spoof L, Morrison LF, Codd GA (2006) Production of antibodies against microcystin-RR for the assessment of purified microcystins and cyanobacterial environmental samples. Toxicon 48:295–306

Yuan M, Carmichael WW (2004) Detection and analysis of the cyanobacterial peptide hepatotoxins microcystin and nodularin using SELDI-TOF mass spectrometry. Toxicon 44:561–570

Yuan M, Carmichael WW, Hilborn ED (2006) Microcystin analysis in human sera and liver from human fatalities in Caruaru, Brazil 1996. Toxicon 48:627–640

Zhao Y, Xie P, Tang R, Zhang X, Li L, Li D (2008) In vivo studies on the toxic effects of microcystins on mitochondrial electron transport chain and ion regulation in liver and heart of rabbit. Comp Biochem Physiol C Toxicol Pharmacol 148:204–210

Zhao Y, Xie P, Zhang X (2009) Oxidative stress response after prolonged exposure of domestic rabbit to a lower dosage of extracted microcystins. Environ Toxicol Pharmacol 27:195–199

Zurawell RW, Chen H, Burke JM, Prepas EE (2005) Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. J Toxicol Environ Health 68:1–37

Arthrospira (Spirulina)

25

Claudio Sili, Giuseppe Torzillo and Avigad Vonshak

Contents

Summary

The successful commercial exploitation of *Arthrospira* because of its high nutritional value, chemical composition and safety of the biomass has made it one of the most important industrially cultivated microalgae. Knowledge of its biology and physiology, which is essential for understanding the growth requirements of this alkaliphilic organism, has been used in developing suitable technologies for mass cultivation. The relationships between environmental and cultural factors, which govern productivity in outdoor cultures, are discussed in connection with growth yield and efficiency. The response of *Arthrospira* and its modification under stress is described, together with the strategy of osmotic adjustment and the mechanism of internal pH regulation to alkalinity. The metabolic plasticity of the response to disparate environmental stimuli is demonstrated in the natural environment, but is also well-expressed in the maintenance of high productive monoculture in intensive outdoor cultivation systems.

While the confused taxonomy of *Arthrospira* and its relationship with *Spirulina* has been resolved by study of the ultrastructural feature of trichomes and 16S rRNA sequence analysis, the problem of species definition is still ongoing. However, molecular methods such as total DNA restriction profile analyses of a wide range of strains are helping to resolve this.

25.1 Introduction

This account needs to start with a taxonomic comment. Ever since *Arthrospira* was first reported in 1852 by Stizenberger, many species of this genus of helically coiled cyanobacteria have been described and isolated. However, its classification has long been a source of confusion. Geitler (1925) invalidated the genus *Arthrospira* in his revision of the Cyanophyceae and included all regularly helically coiled Oscillatoriales without firm sheaths in the previously described

C. Sili (✉) • G. Torzillo
Istituto per lo Studio degli Ecosistemi, CNR,
Via Madonna del Piano, 10, 50019, Sesto Fiorentino, Firenze, Italy
e-mail: sili@ise.cnr.it; torzillo@ise.cnr.it

A. Vonshak
Microalgal Biotechnology, The Jacob Blaustein Institute
for Desert Research, Ben-Gurion University of the Negev,
Sede Boker Campus, Beersheba, 84990, Israel
e-mail: avigad@bgu.ac.il

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genus *Spirulina* Turpin 1829. Most authors followed Geitler for a long while, but increasingly researchers realized that genera are distinct and returned to using two names. The evidence supporting this was summarized by Castenholz (2001) and Komárek and Anagnostidis (2005). Many species currently listed as *Spirulina* should therefore be re-included in *Arthrospira* and these include all those grown commercially and sold as Spirulina. This material is now known so widely under this name that it seems inevitable that the name will persist; however, it should be written as Spirulina or spirulina i.e. no italics.

Arthrospira has been reported to exist in environments varying in their osmoticum, temperature and salt concentrations, most being of high alkalinity (Iltis 1969a, b; Busson 1971). Filaments of (true) *Spirulina* also occur in many of these environments, but apparently never forming the blooms that often occur with *Arthrospira*.

There is great interest in past and present use of *Arthrospira* as a food, though nowadays mostly as a "health" food. Dangeard (1940), Brandily (1959) and Léonard and Compère (1967) all described how African tribes living along Lake Chad collect this alga. The biomass is harvested from waterbodies near the lake and sun-dried on the shores to produce a hardened dark cake called "dihé", which is broken into small pieces and used in different forms by the local populations as part of their daily diet. At about the same time, *Arthrospira* was also recorded in the water of Lake Texcoco, Mexico. Here, it had been used as food by the natives living in the area (Clément 1968). Travellers to Mexico during the sixteenth century described how the Aztecs used a soft a blue-green material, harvested with fine nets from the lake, for making a kind of bread called "tecuitlatl" (Ciferri 1983). It is striking that these two human populations, living far apart, discovered the nutritional value of *Arthrospira* independently.

Later, attention was refocused on "Spirulina" by the pioneering work of the Institute Française du Pétrole on cyanobacterial blooms in the evaporation ponds of the industrial soda production facility at Lake Texcoco near Mexico City. This led to the first detailed study of the growth requirements and physiology of *Arthrospira*. The Ph.D. research of Zarrouk (1966) was the basis for establishing the first large-scale production plant. The work was followed up by several groups in Italy, France and Israel and summarized in a review by Ciferri (1983). The subsequent extensive research on cell biology, biochemistry and biotechnology has been reviewed in books edited by Vonshak (1997a), and Richmond (2004a). This chapter provides a perspective on the biology and biotechnology of the two most important species of *Arthrospira* utilized for commercial mass cultivation, *A. maxima* and *A. fusiformis* (usually reported as Spirulina maxima and Spirulina platensis, respectively).

25.2 Morphology

The main morphological feature of *Arthrospira* is the typical arrangement of its multicellular cylindrical trichomes in an open helix usually of relatively large diameter, sometimes attenuated at the ends, and with evident cross-walls (Fig. 25.1). In contrast, *Spirulina* presents a screw-like trichome, generally with almost closed, uniform and narrow diameter screws (0.5–3 μm), cells with cross-walls usually invisible at light microscope, without gas vacuoles and with prominent granules (Fig. 25.2). The trichomes of *Arthrospira* are composed of cylindrical cells that undergo binary fission in a single plane perpendicular to the main axis. Trichome elongation occurs through multiple intercalary cell division along the entire filament. Multiplication occurs only by fragmentation of a trichome, usually in correspondence of a necridial cell (Fig. 25.3). The mechanism, has been described in detail for both *A. maxima* and *A. fusiformis* by Tomaselli et al. (1981). It consists in the destruction of an intercalary sacrificial cell (necridium) that first becomes colourless and finally biconcave due to the collapse of the lateral septa. However, the presence of necridia become less evident when cultures are subject to fast mixing as it occurs in mass cultures. In contrast, the fragmentation of *Spirulina* trichomes occurs always without the production of necridic cells (Komárek and Anagnostidis 2005) (Table 25.2).

The trichome width of *Arthrospira* populations sampled from nature ranges from about 2.5 to 16 μm, while the helix pitch typically ranges from 0 to 80 μm and its diameter from 15 to 60 μm. The dimensions and other morphological features of *A. fusiformis* (i.e. the well-known commercial Spirulina platensis) not only vary markedly between populations, but also within one population (Fig. 25.4). Both under laboratory and mass cultivation conditions, the helix architecture (pitch and diameter) is highly dependent on growth and environmental conditions. Observations on *Arthrospira fusiformis* by the authors have shown that morphological variability and trichome motility are especially evident during the first weeks following trichome isolation, and that this is usually maintained for some years in laboratory liquid culture (Fig. 25.5). In contrast, morphological variation in *A. maxima* following its isolation is much less marked (Fig. 25.6). Both *A. fusiformis* (Fig. 25.7) and *A. maxima* typically have abundant gas vacuoles, which help to position the organism in the water column. They have an important role in the harvesting of the bloom by African people who utilize *A. fusiformis* for the preparation of *dihè* (Abdulqader et al. 2000).

The sheath of *Arthrospira* is usually absent in planktonic populations. Where a sheath is present, it is colourless, tube-like, adjacent to the trichome, open at the ends and contains only one trichome (Komárek and Hauer 2011). In contrast, a

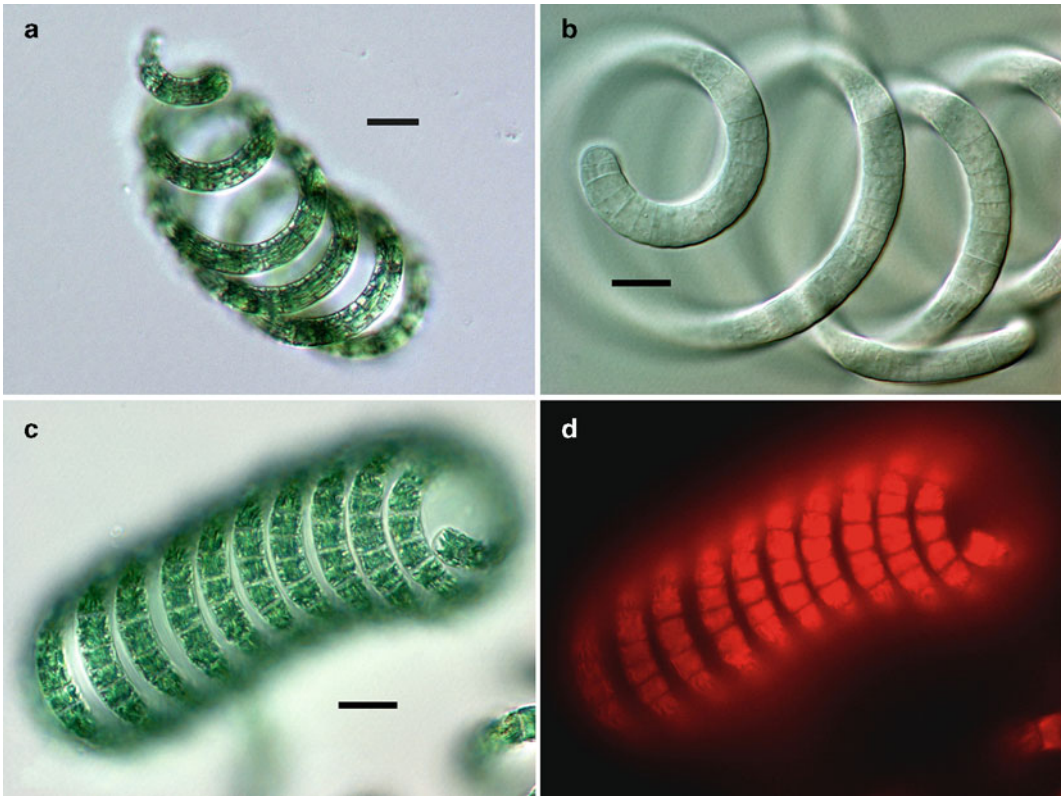

Fig. 25.1 Morphological aspects of the evident cross-walls in *Arthrospira fusiformis* isolated from soda Lake Kailala (Chad). (**a**) Fresh clonal trichome grown in laboratory; (**b**) wild trichome fixed in formaldehyde (2%); (**c**) fresh clonal trichome grown in mass culture; (**d**) autoflorescence of trichome shown in (**c**). Bar marker = 10 μm (Photos C. Sili)

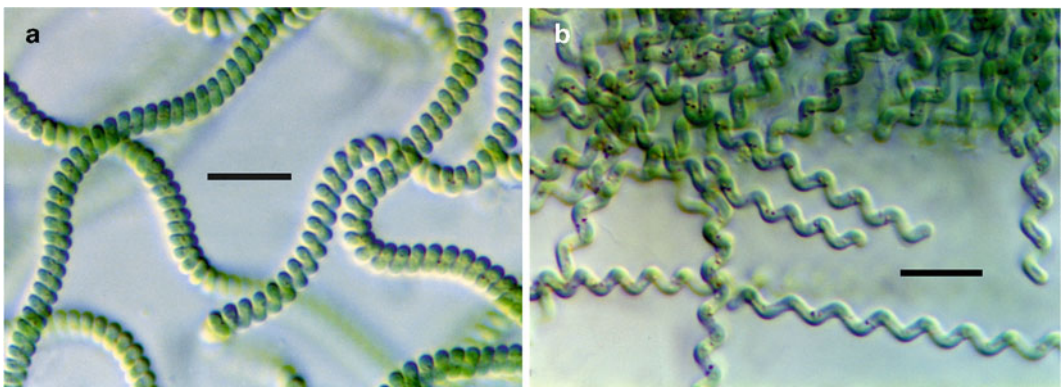

Fig. 25.2 Clonal trichomes of +/− regularly screw-like coiled in (**a**) *Spirulina subsalsa* and (**b**) *S. major*. Note the absence of gas vacuoles and visible cross-walls. Bar marker = 10 μm (Photos C. Sili)

considerable production of mucilage can be formed by a strain of *A. fusiformis,* which was isolated from Lake Kailala, on agar plates, particularly if the material starts to dry out (authors, unpublished data). Under such conditions, cocoon-like shapes, which are generally immersed in a polysaccharidic matrix, with two or more entangled trichomes inside their capsular investment, start to increase (Fig. 25.8a). In some cases, it is possible to observe a single wide mucilaginous envelope surrounding each trichome (Fig. 25.8b). The rupture of the capsule causes the release of 2–4 trichomes (Fig. 25.8c). The polysaccharide slime produced by *A. fusiformis* on agar plates can be dense (Fig. 25.8d). It seems likely that these

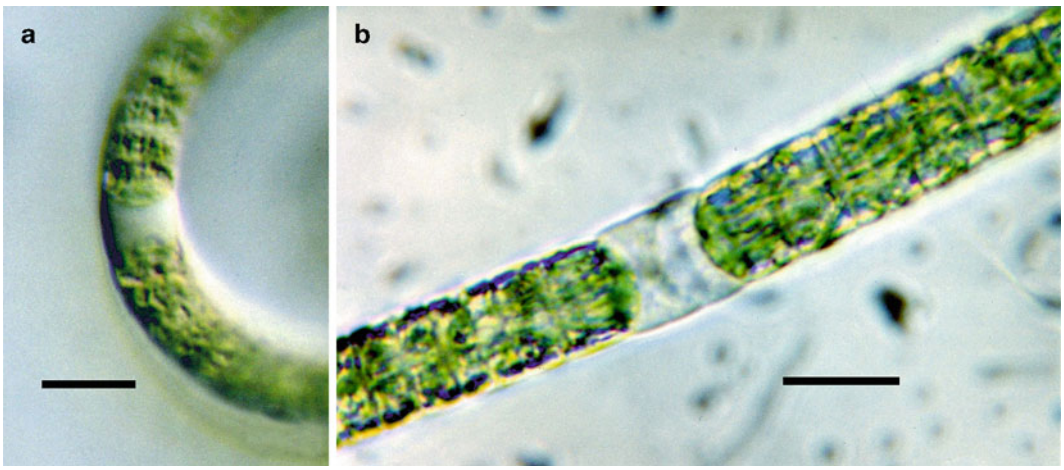

Fig. 25.3 Necridic cell formation in clonal: (**a**) *Arthrospira fusiformis* coiled trichome; (**b**) *A. maxima* straight trichome. Bar marker = 10 μm (Photos C. Sili)

structures are an adaptation to cope with adverse environmental conditions, such as those in the semi-dry zones at the edge of the small alkaline lakes with *A. fusiformis* blooms, which are often subject to rapid and extensive draining with the onset of the dry season.

The effect of temperature on filament structure was studied by Van Eykelenburg (1979), who subsequently described the rapid reversible change from the helix to the spiral shape on solid media (Van Eykelenburg and Fuchs 1980). Although, the simultaneous presence of straight and helicoidal forms of *A. fusiformis* and *A. maxima* (Fig. 25.9) has been observed frequently in the laboratory and in mass cultures, the trigger factor and the mechanism for this morphological change still remain obscure. A detailed study of the effect of physical and chemical conditions on the helix geometry of *Arthrospira* was done by Jeeji Bai and Seshadri (1980) and Jeeji Bai (1985). They reported the occurrence of the straight or nearly straight spontaneous culture variants also observed by Lewin (1980). However, there are considerable differences in the degree of coiling between different strains of the same species and within the same strain, the helical shape of the trichome is regarded as a peculiar property of the genus. Once a strain has converted to the straight form, either naturally or due to physical or chemical treatments, it does not usually revert back to the helical form. Jeeji Bai (1985) suggested that this may be due to a mutation affecting some trichomes under certain growth conditions. The common occurrence of straight trichomes in mass cultures of *Arthrospira* also suggests that the helical character may be carried on plasmids, but no one has yet demonstrated the existence of plasmids in *Arthrospira*. It has been reported that helical shape is related to protection of the organism against photolysis (Fox 1996). Wang and Zhao (2005) observed that the helical filaments originated from the linear filaments 9 years old obtained with ionizing radiation. Further studies revealed that under growth conditions, the linear trichomes could spontaneously revert to the helical trichomes with the same morphology as the original ones. These authors also observed, there were significant differences in morphology, ultrastructure, physiology, biochemistry, and genetic characteristics between the linear trichomes and the original or reverted ones, but no differences were found between the original (helical) and the reverted trichome, indicating that both linearization and reversion the helical shape of *Arthrospira* trichomes were due to genetical variation. Occasional helical trichomes in cultures of *Arthrospira fusiformis* strain D885/S which is a straight form, have been also observed by Mühling et al. (2006). However, in all cases the helical trichomes were outcompeted by straight ones and disappeared after subsequent subculturing. In contrast, Noor et al. (2008) found that a stock culture of *Arthrospira* (Spirulina) maintained it helical structure after 17 years of subculture, while filaments in pilot ponds lost their helical structure. They concluded that the prolonged period of acclimation to adverse climatic conditions outdoors, may cause reversion of coiled filaments to straight ones, and that this fact supports the idea that straight filaments may have a higher survival capacity. However, biochemical composition and nutritional properties were not affected by the shape of the filaments (Noor et al. 2008; G. Torzillo, unpublished).

Interestingly, Mussagy et al. (2006), starting from a culture of hormogonia of the straight trichomes, obtained one with a mix of loosely-coiled and straight ones. Finally, Hongsthong et al. (2007), using a proteomic approach, concluded that the linear morphology observed both in the laboratory and in industrial production ponds was possibly induced firstly by environmental stresses, such as oxygen level, carbon dioxide level, nutrient availability and light, and secondly by the change in a cell shape determination mechanism. This study

Table 25.1 Morphology of *Arthrospira* species based on Komárek and Anagnostidis (2005) and Komárek and Hauer (2011)

Species	Trichome width (μm)	Cell length (μm)	Coils (μm)		Helix shape	Gas vacuoles	Morphology of trichome end
			Width	Height			
Types without gas-vacuoles (forming mats)							
A. balkrishnanii Kamat 1963	3.5–4 (5)	0.8–1.2	6–7	12–15		–	Rounded. no calyptra
A. desikacharyensis Vasishta 1962		0.85–1.7	51–6.8	10.2–11.5	Regularly loosely spiral coils	–	Rounded
A gigantea (Schmidle) Anagnostidis 1998	(2.5) 3–4		(8) 11–16	7–12 (15)	Regularly coiled	–	+/– conical
A gomontiana Setchell 1895	2.5–3	+/– isodiametric	6–6.5	15–19		–	
A. jenneri Stizenberger ex Gomont 1892	(3.7) 4–6 (8)		(7.4) 8–15 (17)	(9.2) 12–25 (31)	Regularly screw-like coils	–	Rounded, no calyptra
A. massartii Kufferath 1914	5–6				Loosely screw-like	–	Rounded conical
A. pellucidis Wang 1933							
A. platensis (Nordstedt) Gomont 1892	(4) 6–7 (9?)	Nearly isodiametric	(20?) 26–36	(24?) 30–57	+/– regularly loosely spiral coils	–	Rounded or flat calyptra
A. santannae Komárek et Komárková-Legnerová 2006	2.8–3.8	+/– isodiametric	12.5–18.5 (loose) 8.8–10.8 (dense)	20–40 (dense) 5.5–5.8 (loose)	+/– densely or loosely coil	–	Rounded, no calyptra
A. skujae Magrin, Senna et Komárek 1998	(2.7) 3.1–4.3	3.1–9.3	7.4–11.8	7–13	Regularly screw-like coil	–	Rounded
A. tenuis Brühl et Biswas 1922	About 2	2–3	20–35			–	
Types with gas vacuoles (planktonic, solitary trichomes)							
A. argentina (Frenguelli) Guarrera et Kühnemann 1949	9–10.5		33–49	63–75		+	
A. fusiformis (Voronichin) Komárek et Lund 1990	(3.4)5–9 (11?)	2–3 × shorter than wide	15–50	0–80	Regularly screw-like coiled intensely narrowed or widened towards ends	+	Rounded
A. indica Desikachary et Jeeji Bai 1992	8–9	1/3–1/2 as long as wide	16–39	16–66	Spirally coiled and distinctly narrowed towards ends	+	Conical calyptra
A. ghannae Drouet et Stirickland in Drouet 1942	3–5.2		20–22	20–35	Regularly loosely screw-like coils	+	Slightly attenuated end and capitate
A. joshii Vasishta 1963	4.2–5.1	2.7–3.4	6.8–7.6	17–19.2	Regularly screw-like coils, not attenuated towards ends	+	Rounded, no calyptra
A. maxima Setchell et Gardner in Gardner 1917	(6.5) 7–9 (10)	5–7	40–60	70–80	Regularly loosely screw-like coiled, gradually slightly attenuated at ends	+	Rounded with thickend outer cell wall

Fig. 25.4 Morphological variability in *Arthrospira fusiformis* in two soda lakes at the irregular north-east fringe of Lake Chad. (**a**, **b**, **c**) Wild fresh trichomes from Lake Kailala; (**d**, **e**, **f**) wild trichomes fixed in formaldehyde (2%) from Lake Kossorom. (**f**) Shows that occasional ± straight trichomes may also be present in a wild sample. Bar marker = 40 μm (Photos C. Sili)

was the first to show evidence at the protein level that could explain this morphological transformation in *Arthrospira*.

The effect of solar UV radiation on morphology on *Arthrospira* was studied by Wu et al. (2005) using an indoor grown strain, which had not been exposed to sunlight for decades, and an outdoor-grown strain grown under sunlight for decades. The experiments confirmed what had already observed by Jeeji Bai and Seshadri (1980) that the self-shading effect produced by compression of the spirals over adaptive time scales seem to play an important role in protecting this species against deleterious UV radiation. Gao et al. (2008) described interactions between temperature and UVR on the morphology of *A. fusiformis* and found that UVR caused a breakage of the spiral structure at 15°C and 22°C, but not at 30°C. *A. fusiformis* was able to modify its spiral structure by increasing helix tightness at the highest temperature (30°C). PAR has been found to cause a decrease in the helix pitch, while UVR increased the extent to which helices are compressed (Ma and Gao 2009). In general it seems that artificial laboratory conditions favour the development of straight forms. After 8 years of repeated transplants in liquid culture, two *A. fusiformis* strains (Kailala and Kossorom) had about 30% had straight trichomes (Fig. 25.10a), 50% with loosely-coiled trichomes (Fig. 25.10b), while only 20% maintained their original or an elevated degree of helicity (Fig. 25.10c) (C. Sili, unpublished data).

A survey of the morphological characters of 36 clonal axenic strains of cultivated *Arthrospira* carried out by Mühling et al. (2003) showed that of the 34 with helical trichomes, five were right-handed and 29 left-handed. After 1 year of repeated subculture, the orientation of one helical strain had changed from right to left-handed, suggesting a probable genetic shift. In addition, a temperature shift from 30°C to 34°C for 7 days led to a change in orientation in three strains, which was reversible if the temperature shift was reversed. The fact that the orientation of trichome helix can be reversed by genetic drift and by environmental factors such as temperature upshift and mechanical stress demonstrates

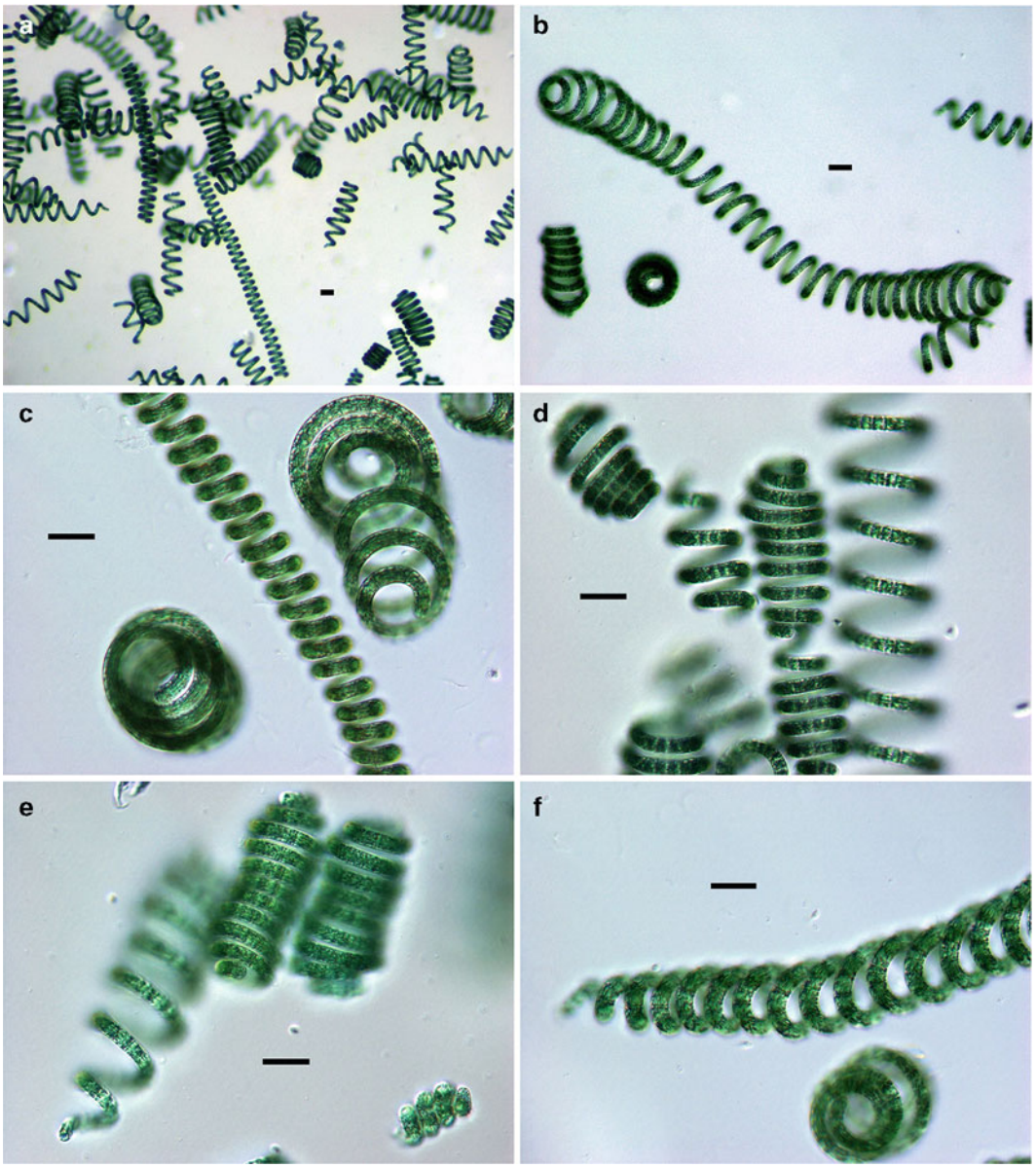

Fig. 25.5 Morphological variability in fresh clonal trichomes of *Arthrospira fusiformis* isolated from soda Lake Kailala (Chad) and grown in the laboratory. Bar marker = 20 μm (Photos C. Sili)

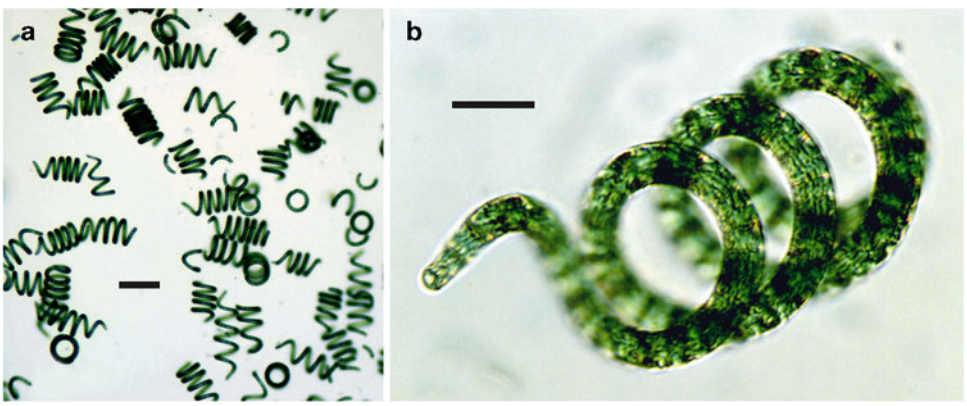

Fig. 25.6 (**a**) Morphological aspects of regular clonal trichomes of *Arthrospira maxima* isolated from the solar Lake Texoco evaporator. (**b**) Typical trichome regularly screw-like coiled with gradually attenuated cells diameter at the ends. Bar markers correspond to 40 μm in **a** and 20 μm in **b** (Photos C. Sili)

Fig. 25.7 (**a**) Clonal trichomes of *Arthrospira fusiformis* isolated from the soda Lake Kailala (Chad) with a typical spiral markedly narrowed towards the ends. (**b**) *Dark-field photo* showing the abundant gas vacuoles. Bar marker = 20 μm (Photos C. Sili)

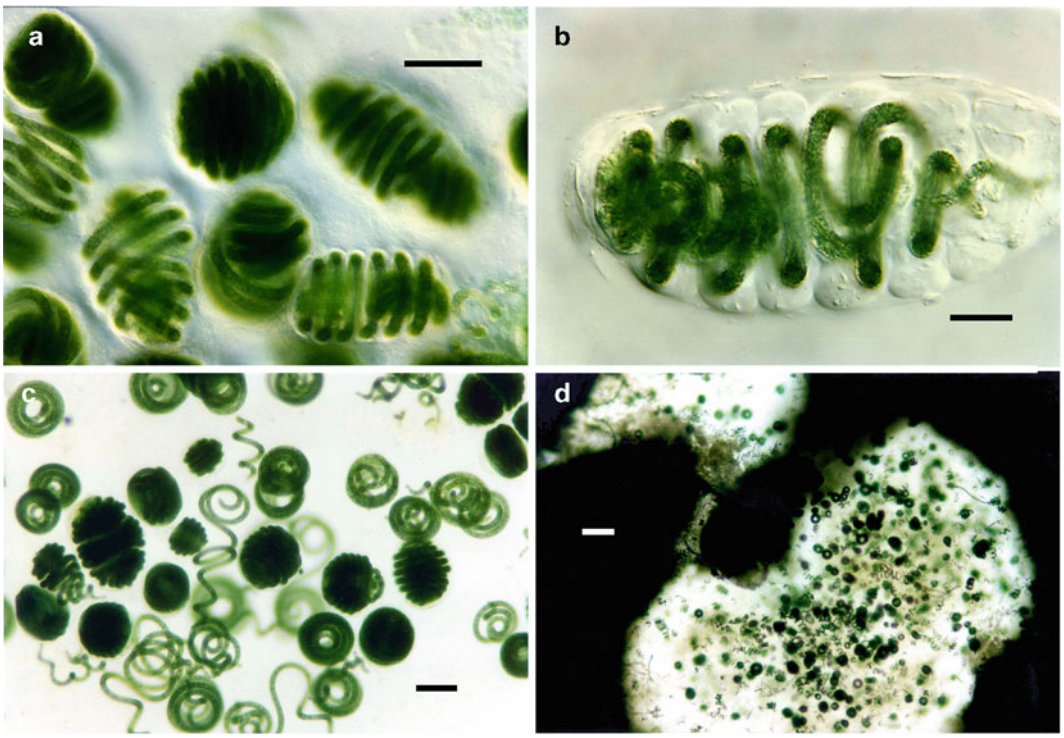

Fig. 25.8 Morphology of *Arthrospira fusiformis* grown on agar Petri dishes. (**a**) Cocoon-like shape immersed in a polysaccharide matrix, with two or more internal entangled motile trichomes; (**b**) two single trichomes surrounded by mucilaginous envelopes that, in turn, are surrounded by thick capsules; (**c**) clusters of trichomes of a typical cocoon-like shaped after their release as single filaments; (**d**) cocoon-like shaped and free trichomes immersed in a dense slime of polysaccharides negatively stained with Indian ink. Bar markers correspond to 40 μm in (**a**) and (**c**), 20 μm in (**b**) and 100 μm in (**d**) (Photos C. Sili)

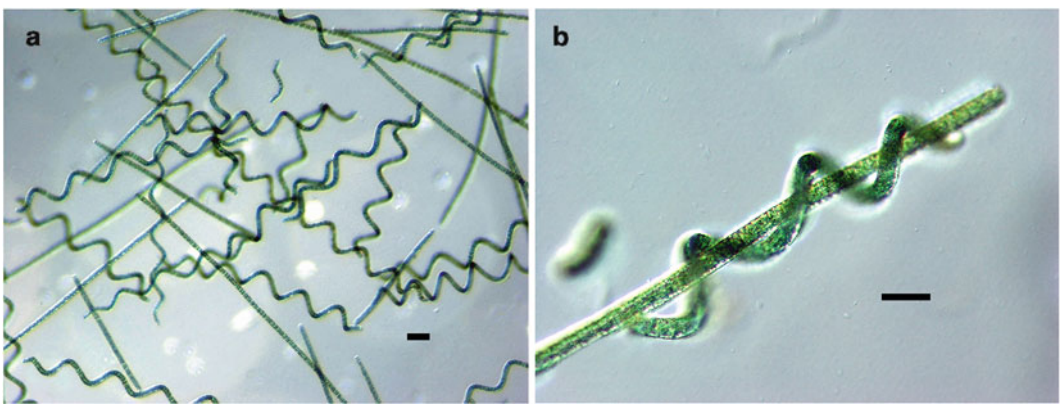

Fig. 25.9 Straight and loosely-coiled clonal trichomes of *Arthrospira maxima* isolated from Lake Texoco (Mexico). Bar markers: A = 40 μm, B = 20 μm (Photos C. Sili)

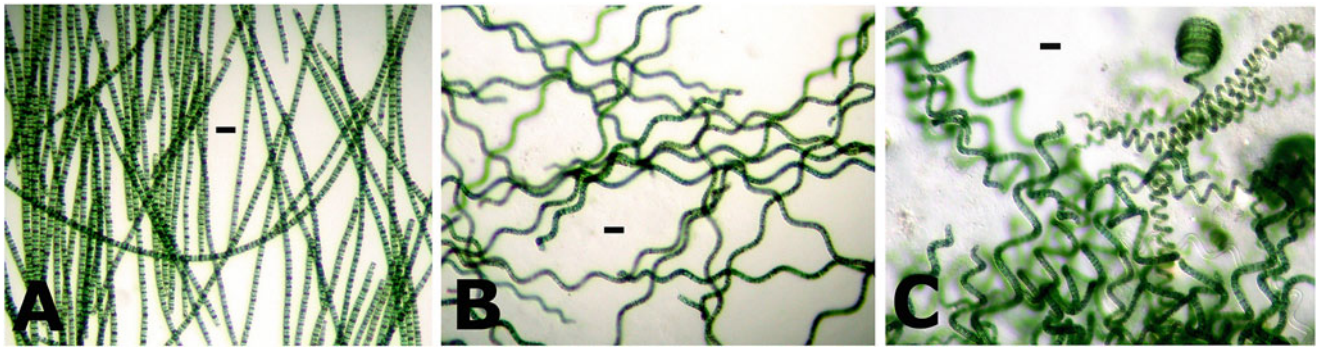

Fig. 25.10 *Arthrospira fusiformis* isolated from Lake Kailala (Chad) after 8 years of repeat transplants in liquid culture: (**a**) clonal straight trichomes; (**b**) loosely coiled trichomes; (**c**) coiled trichomes. Bar marker = 20 μm (Photos C. Sili)

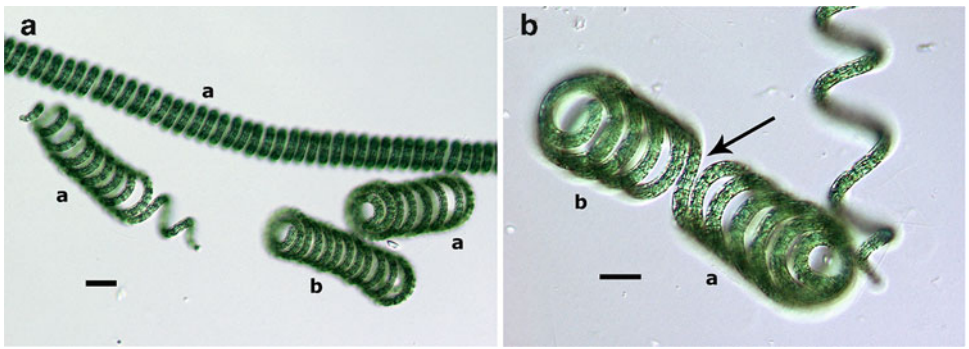

Fig. 25.11 Examples of different helix orientations observed in *Arthrospira fusiformis* cultures. (**A**) left-handed helices, (*a*); right-handed helices (*b*). (**B**) Example of reversal helix orientation due to mechanical stress; *arrow* indicates the position on the trichome where the helix orientation reversed from left-hand (*a*) to right-hand (*b*). Bar marker = 20 μm (Photos C. Sili)

that care is needed in using this character for taxonomic purposes (Mühling et al. 2003). Occasionally, reversed forms from left-to- right handed orientation have been observed in our *Arthrospira fusiformis* laboratory and mass cultures (Fig. 25.11).

Sinking velocity is markedly influenced by the shape of trichomes. If a planktonic species evolves towards a decreasing sinking velocity, it has three options: (i) it may decrease its body size (but this could increase the risk of being grazed by zooplankton); (ii) it may decrease its specific gravity (e.g. increasing gas vacuole density) or reducing the amount of ballast substances (e.g. carbohydrate); (iii) or may increase its form resistance. Padisák et al. (2003) investigated the sinking velocities of different PVC models of phytoplankton organisms. The sinking velocity was related to dimensionless form resistance factor (Φ). It was observed that Φ decreased in coiled shaped organisms, and increased with the straight ones. As a result coiled forms tend to sink at a faster speed than straight ones. These findings agree with those made by Booker and Walsby (1979) on *Anabaena flos-aquae*. They observed that helical filaments of all length classes sank significantly faster than straight ones of corresponding length. In the case of straight filaments there was a slight progressive increase in mean sinking speed with increasing length helical filaments though up to 10–20 cells. The authors concluded the advantage of straight filaments could not be in conferring a higher growth rate, but it might be in resisting predation or affecting the rate of sinking and floating. Similar conclusions were drawn by Mühling et al. (2003). These authors reported that in one example of grazing by large ciliate, the ciliate had to make a rotatory motion almost following the coil of the *Arthrospira*, with up to 10 coils showing inside the ciliate. It was also suggested that, perhaps the reversal of helix orientation at intervals within a particular ecosystem in response to shifts between grazers or changes within populations of one particular grazer.

The ultrastructural cell organization of *Arthrospira* is typical of prokaryotes, with a lack of membrane-bound organelles (Van Eykelenburg 1979; Vonshak and Tomaselli 2000). The thin cell wall has four layers, with an easily detectable electron-dense layer corresponding to the peptidoglycan layer (Van Eykelenburg 1977). The regularly spaced cross-walls divide the trichome into cells connected by plasmodesmata. Cross-walls are formed by centripetal growth and extensions of both the peptidoglycan and the more internal layer of the cell wall toward the centre of the cell. The cell

has a number of inclusions mostly corresponding to the typical ones for cyanobacteria described in previous books (Fogg et al. 1973; Carr and Whitton 1973, 1982). Thylakoid membranes with phycobilisomes are arranged in parallel bundles. The other main subcellular inclusions are (in addition to carboxysomes, ribosomes and DNA fibrils) gas vacuoles, polyglucan granules, polyphosphate granules and large cyanophycin granules. The extent of development of the last three depends on the nutrient status of the trichome; for instance N-rich conditions favour cyanophycin formation.

Structures like the cylindrical bodies reported for some other members of the Oscillatoriaceae are often present. These inclusions, whose explanation is still unknown, are lacking in *Spirulina* (Tomaselli et al. 1996; Tomaselli 1997). Studies carried out by Palinska and Krumbein (2000) on the peptidoglycan cell wall of cyanobacteria confirmed the observations made by Guglielmi and Cohen-Bazire (1982) concerning different perforation patterns of *Spirulina* and *Arthrospira*, which were used as a criterion in order to separate them clearly into two different genera. Spiller et al. (2000), using an x-ray microscope, showed that the cell end walls were clearly visible in *A. fusiformis*, and that they crossed the filament at intervals of about 2–3 μm. The images also revealed two interesting structures characterised by a spherical shape, as well as a looping strand that resembled beads on a string.

25.3 Taxonomy

As discussed in the introduction section, the question of the taxonomy and nomenclature of *Arthrospira* and *Spirulina* was debated for almost the entire twentieth century. Several questions have been solved only in recent decades with the help of electron microscopy, biochemical analysis, and recently, thanks to the support of molecular analysis, within the context of what is commonly referred to as a "polyphasic approach". The genus *Arthrospira* has now become universally accepted, at least by taxonomists, as the name for the organisms used in mass culture. With the aim of further clarifying the systematic position of the *Arthrospira* spp. commonly used for mass culture operations (basically speaking, *A. maxima* and *A. fusiformis*), Komárek and Anagnostidis (2005) and Komárek and Hauer (2011) have recently urged a definitive abandoning of the commercial name *Spirulina* (*Arthrospira*) *platensis*, recommending that it be replaced with the taxonomically-correct name of *A. fusiformis.*

The long story concerning the name *Spirulina* started about 130 years ago, when Wittrock and Nordstedt (1884) reported the occurrence in a stagnant pond near Montevideo of a blue-green alga with helical shaped filaments, which they described as "*Spirulina jenneri* f. *platensis*", even though it possessed septa. At that time the genus *Spirulina* was considered to be unicellular. Some years later, Stizenberger (1852), who had observed septa in some helically coiled oscillatoriacean forms, proposed that the forms, with a multicellular structure, be included in a new genus, *Arthrospira*. This distinction was confirmed later by Gomont (1892) in his taxonomic study on the Oscillatoriaceae. He left the apparently aseptate forms (*trichomata unicellularia*) in *Spirulina* and placed the septate forms (*trichomata pluricellularia*) in *Arthrospira* Stizenberger 1852.

However, Geitler's revision (1925, 1932) of the Cyanophyceae re-unified the members of these two genera in *Spirulina* Turpin 1829. He based classification on the close similarity in morphology and ignored the presence of septa and thus made no distinction between the forms previously recognized as separate genera. The genus was thus divided by Geitler into two subgeneric taxa (Section I. Arthrospira and Section II. Euspirulina) on the basis of the criterion originally used by Stizenberger (1852) to separate the genera *Spirulina* and *Arthrospira* i.e. visible or non-visible cross-walls. The forms with septa that were easily observable under a light microscope were classified in Section Arthrospira and those with unlikely or only artificially observable septa (after trypsin treatment or staining with neutral red) in Section Euspirulina.

In accordance with those authors who sustain that many aseptate forms are only apparently so, including species that reveal septa only after proper chemical treatment and staining, Desikachary (1959) returned to a separation of the two genera: *Spirulina*Turpin with invisible or unicellular cells of trichome, and *Arthrospira* Stizenberger with cells of the trichome clearly visible. Subsequently, Bourrelly (1970), by following a radical taxonomy attribution, merged *Spirulina* and *Arthrospira* in *Oscillatoria,* and remarked that new understanding of cell colouration made it possible to show also the otherwise invisible septa of *Spirulina*. Hindák (1985) assigned all planktonic forms of *Arthrospira* (including those previously described as "*S. platensis*") in alkaline saline lakes to "*S. fusiformis*" Voronichin 1934 (synonyms *A. maxima* Setchell et Gardner 1917, in Gardner 1917, and *A. geitleri* De Toni 1935), since, in agreement with Fott and Karim (1973), he considered "*S. platensis*" to be a benthic species.

In order to distinguish *Spirulina* and *Arthrospira* in Bergey's Manual of Systematic Bacteriology, Castenholz (1989) suggested the use of three features of helically coiled trichomes: (1) degree of inclination of the pitch of trichome helix (from transverse axis); (2) aspect and visibility (LM) of cross-walls between the cells in the filament; (3) distribution (EM) of junctional pores in the cell wall. Thus *Arthrospira* can be differentiated from *Spirulina* on the basis of the degree of inclination of the pitch of the trichome helix, which forms an angle >45° from the transverse axis, the presence of easy visible septa and the distribution of junctional pores in one circular row around the cross-walls. In 2001, with the Second

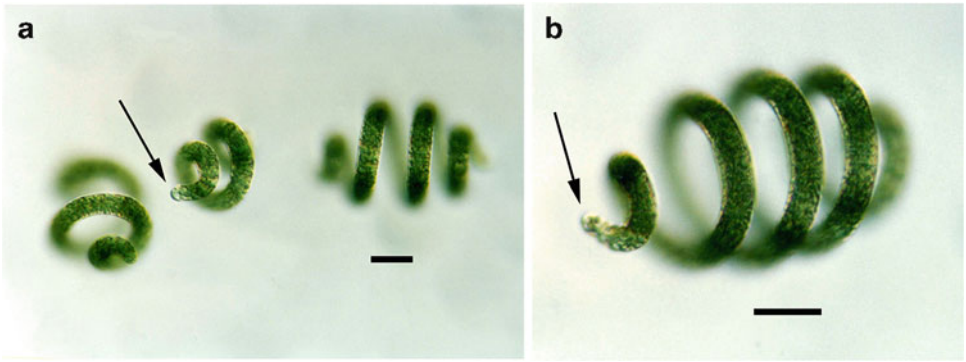

Fig. 25.12 Clonal trichomes of *Arthrospira indica* isolated from Lake Lonar. *Arrows* indicate the presence of a calyptra. Bar marker = 20 μm (Photos C. Sili)

Edition of Bergey's Manual of Systematic Bacteriology, Castenholz updated the key to separate *Spirulina* from *Arthrospira*. The two genera were distinguished according to the trichome helix, cross-walls whether invisible or visible, and cell diameter 2–4 μm in *Spirulina* versus typically 6–12 μm in *Arthrospira*.

An important revision of the taxonomy and nomenclature for "*Spirulina platensis*", "*S. maxima*" and "*S. fusiformis*", was suggested by Komárek and Lund (1990) who placed the two taxa ("*maxima*" and "*fusiformis*") within the planktonic forms (i.e. with gas vacuoles). Furthermore, they suggested that these two taxa, which may represent two species (*A. fusiformis* Komárek et Lund and *A. maxima* Setchell et Gardner), have different geographical distributions. Hence, they considered *A. maxima* to be pantropical, while *A. fusiformis* was limited to Africa and tropical and central Asia. Moreover, for the first time they considered *A. platensis* (without gas vacuoles, similar to *A. jenneri*) to be a periphytic and benthic species that is essentially restricted to South America.

Subsequently, Desikachary and Jeeji Bai (1992) and Jeeji Bai (1999) proposed that all the calyptrate forms of *A. fusiformis* should be included in the new species *A. indica* Desikachary. This implied that the thickening of the apical cell wall (calyptra) should be considered a significant taxonomic feature (Fig. 25.12). Komárek and Anagnostidis (2005) concluded that *Arthrospira* has solitary and free-floating trichomes in the planktonic forms of subtropical and tropical saline lakes (*A. maxima* and *A. fusiformis*), while freshwater benthic forms were united in a fine, mostly slimy, thallus in *A. platensis* and *A. jenneri*. These authors placed *Arthrospira* to the family Phormidiaceae (subfamily Phormioideae) and recognized 17 species worldwide according based on phenotypic markers. The main morphological features of species without (i.e. benthic) gas vacuoles (benthic) or with (i.e. planktonic) gas vacuoles, as distinguished by Komárek and Anagnostidis (2005) and more recently by Komárek and Hauer (2011), (http://www.cyanodb.cz). In the light of this new taxonomic grouping, the Table 25.2 provides a summary of the synonyms of different *Arthrospira* species recognised by Komárek and Anagnostidis (2005). However, this classification which is based only on phenotypic aspects needs to be supported, particularly for the cultivated strains, by molecular analysis.

In the past 20 years, the great development of molecular biology allowed to accompany this modern techniques to the taxonomy based only on morphological features in accordance with a combined methodology that Colwell (1970) for the first time indicated as the "polyphasic approach".

Viti et al. (1997), using total DNA restriction profile analysis, carried out on 10 strains belonging to *Arthrospira maxima* and *Arthrospira platensis* (*fusiformis*), found a great consensus between these the genotypic clusters and those grouped using morphological criteria. Subsequently, Scheldeman et al. (1999), by means of phylogenetic study using ARDRA (Amplified Ribosomal DNA Restriction Analysis) of the ITS (Internal Transcribed Spacer) as a molecular taxonomic marker, tested 51 *Arthrospira* cultures that theoretically represented 37 unique genotypes obtained from different culture collections coming from four continents. The studies confirmed a clear separation of *Spirulina* from *Arthrospira* and the presence of two main genotype clusters within *Arthrospira* which were unrelated as far as the geographic origin of the strains, their denomination or their phenotypic features.

In 2002 Manen and Falquet investigated the genetic diversity of 23 natural, cultivated and commercial strains of *Arthrospira* by comparing the genus with 20 other non-*Arthrospira* cyanobacterial strains, utilizing the phycocyanin locus (*cpcB-cpcA*). These studies confirmed once again, that *Arthrospira* is not related to *Spirulina* and for the first time showed that the former genus constitutes a strongly sustained monophyletic group. Furthermore, the three genetically clustered lineages according to which *Arthrospira* was divided confirmed the general mismatch between their geographic origin and morphology. Similar conclusions were reached by Baurain et al. (2002), who found no clear relationships

Table 25.2 Synonyms of main *Arthrospira* species, based on Komárek and Anagnostidis (2005)

Name	Synonyms
Species of saline alkaline waterbodies, planktonic, with gas vacuoles (forming blooms)	
A. fusiformis (Voronichin) Komárek et Lund (1990)	*Spirulina fusiformis* Voronichin 1934;
	Arthrospira platensis (Nordstedt) Gomont sensu Rich 1931, 1932, Thomasson 1960, Léonard and Compère 1967, and later authors;
	Arthrospira platensis (Nordstedt) Gomont f. *minor* Rich 1931;
	Spirulina geitleri f. *minor* (Rich) Fott et Karim 1973;
	Spirulina platensis (Gomont) Geitler sensu Thomasson 1960, Jeeji Bai and Seshadri 1980
	Oscillatoria platensis (Nordstedt) Bourrelly 1970;
	Oscillatoria platensis (Nordstedt) Bourrelly 1970 var. *minor* Rich in Iltis 1970;
	Oscillatoria sp. sensu Iltis 1970;
	Spirulina maxima (Setchell et Gardner) Geitler sensu Fott and Karim 1973
A. indica Desikachary et Jeeji Bai 1992	*Spirulina fusiformis* sensu Jeeji Bai and Seshadri 1980, non Voronichin 1934;
	Arthrospira platensis f. *granulata* Desikachary 1959
A. maxima Setchell et Gardner in Gardner 1917	*Spirulina maxima* (Setchell et Gardner) Geitler 1932;
	Spirulina geitleri De Toni 1935 and sensu Fott and Karim 1973;
	Spirulina platensis (Gomont) Geitler sensu Welsh 1965, and later authors;
	Oscillatoria pseudoplatensis Bourrelly 1970;
	non *Spirulina maxima* Bernard 1909
Species of freshwaters, periphytic and benthic without gas vacuoles (forming mats)	
A. jenneri Stizenberger ex Gomont 1892	*Spirulina jenneri* (Stizenberger) Geitler 1935;
	Oscillatoria jenneri (Gomont) Compère 1974
A. platensis (Nordstedt) Gomont 1892	*Spirulina jenneri* var. *platensis* Nordstedt in Wittrock and Nordstedt 1884;
	Spirulina platensis (Nordstedt) Geitler 1925 and later authors;
	Oscillatoria platensis (Nordstedt) Bourrelly 1970;
	Arthrospira platensis var. *tenuis* (Rao) Desikachary 1959

between ITS clusters and the denomination, morphology and origin of the strains. A phenotypic analysis on 35 (33 helical and 2 straight) axenic and clonal *Arthrospira* strains was performed by Mühling et al. (2006). The strains included 28 features, 14 of which were morphological, 1 ultrastructural, 7 physiological, and 6 biochemical. This study reported two phenotypic clusters that had a high correlation to the characters describing the helical trichome shape: cluster I had a highly variable, always irregular, trichome helix that was dumbbell-shaped, or a fusiform trichome helix with a fast-diminishing helix attenuation toward the apices (*A. maxima*, *A. fusiformis*, *A. indica*), and cluster II with a regular trichome helix and fast-diminishing helix attenuation toward the apices (*A. platensis*). These two clusters compared to the molecular ones of the same strains reported by Scheldeman et al. (1999) and Baurain et al. (2002) showed a high rate correlation.

Thirty-three new strains of *Arthrospira* isolated from plankton sampled in Mexico, East Africa and India were investigated and compared with 53 strains or samples of earlier studies (Dadheech et al. 2010). The study included morphological features and molecular phylogenetic analyses on the basis of nucleotide sequences of ITS between 16S rRNA and 23S rRNA genes and partial sequences of beta and alpha subunits including in their genes spaces (*cpc* BA-IGS) of phycocyanin operon. It proved possible to divide the 53 strains into two main clusters: cluster I comprising sequences of American strains, and cluster II containing the sequences of the strains originating from Asia and Africa. Both genetic regions of the strains in this study showed a significant sequence divergence among *Arthrospira* strains from East Africa, India and Mexico, indicating possible distinct evolutionary lineages. It was concluded that there are deep genotypic divergences between Mexican and African/Indian strains of *Arthrospira*, which represent two distinct genotypes, which were also distinguishable on the basis of trichome morphology, and referable to the two tropical main planktonic species *A. fusiformis* and *A. maxima.*

In conclusion, it appears clear that *Arthrospir*a is distinct from *Spirulina* based on both morphological and molecular criteria. However, because of the high morphological plasticity observed particularly with wild and clonal forms (i.e. laboratory and mass culture strains) of *A. fusiformis*, it is sometimes difficult to distinguish the two edible species of *Arthrospira* (*A. fusiformis* and *A. maxima*) by using only a morphological approach. The continuous advancements achieved by molecular analysis, provided that the origin of strain is well known, can represent an important tool that should contribute to better identification of the *Arthrospira* species.

25.4 Occurrence and Distribution

Species of *Arthrospira* have been isolated mainly from alkaline, brackish and saline waters in tropical and semitropical regions (Castenholz 2001; Komárek and Anagnostidis 2005). One of the striking facts about soda lakes is that, despite apparently what might appear unfavourable conditions, these environments are extremely productive because of high ambient temperatures, high light irradiance and unlimited supply of CO_2. Primary production rates up to 10 g m^{-2} day^{-1} have been recorded, which is about one order of magnitude more than most productive streams and lakes in the world, making these the most productive aquatic environments anywhere. A characteristic feature of the majority of soda lakes is the permanent or seasonal coloured blooms of phototrophic microorganisms, as reported in a survey by Grant (2006). Though most records of *Arthrospira* species have been for mass growths in alkaline water, some species have been occur occasionally in freshwaters. Species with apparently more restricted distributions have been reported from puddles (*A. balkrishnaii*), flowing and stagnant fresh water (*A. gigantea, A. jenneri*), springs (*A. massartii*), stagnant water (*A. desikacharyensis*, *A. gomontiana*), stagnant and sulphur-containing water (*A. platensis*), ponds (*A. khannae*), filter beds (*A. tenuis*), reservoirs (*A. santannae*) and tanks (*A. joshi*). Less is known about these species, since they have not been used for mass cultivation.

Among the various species, the most widely distributed, *A. fusiformis* (*A. platensis sensu* Rich 1931), is mainly in Africa (Chad, Kenya, Egypt, Ethiopia, Sudan, Libya, Algeria, Congo, Zaire, Zambia, Mozambique), Asia (Russia, Pakistan, India, Sri Lanka, Myanmar, Thailand) and South America (Uruguay, Peru). *A. maxima* is in Central America (Mexico, California-USA). Until a few years ago, this species was the main component of the phytoplankton of the solar evaporation channels in Lake Texcoco (Mexico), while *A. fusiformis* dominated the alkaline saline lakes of the semi-desert Sudan-Sahel area (Chad) and of the Rift Valleys (mainly Kenya). The frequent occurrence of *A. fusiformis* and *A. maxima* in saline, alkaline, tropical and subtropical waters has led to a widespread, but possibly misleading, impression that these environments are characteristic for the entire genus. The geographical distribution of the main populations of *Arthrospira* is given in Table 25.3. Apparently none have been reported from marine environments. However, when an adequate supply of bicarbonate, N and P, together with a suitable pH and salinity, are provided, *Arthrospira* species can be grown in seawater (Tredici et al. 1986), although the biomass productivity attained was about 36% less than in Zarrouk's medium. The biomass grown in seawater had a reduced protein content (13%).

The most detailed studies on the ecology of *Arthrospira* are those for *A. fusiformis* by Iltis (1968, 1969a, b, 1970, 1971) for tropical African lakes, and that by Busson (1971), who described the ecology of *A. maxima* as a food source in Mexico, together with its past and present use as a food source. Other studies include those of Hindák (1985), Komárek and Lund (1990) and Jeeji Bai (1999).

Most of the waterbodies in which *A. fusiformis* thrives are permanent or temporary saline alkaline lakes (natron lakes). These are scattered in the hollows of the fossil dune system of the Sahara and the Sudan-Sahel areas fed by aquifers and the scanty rain (about 300 mm per year). The salinity of these lakes is highly variable: when evaporation exceeds inflow the salinity rises and leads to an accumulation of salt deposits on the shores (Iltis 1971). The mean water temperature is about 25°C, with oscillations of about ±10°C, although the temperature in shallow lakes may reach 40°C during the warm season. The mean daylight length is about 12 h and solar radiation reaches over 2,000 µmol photon m^{-2} s^{-1}. The disposition and slope of the impermeable clay and water-bearing sandy layers favour subterranean seepage of water away from Lake Chad (Maglione 1969). The relationships between the superficial and the subterranean waters affect both the salinity and the ionic composition. The latter is characterized by prevalence of monovalent ions, with Na^+ much higher than K^+, and by high concentrations of carbonate and bicarbonate, which lead to pH values from 9.5 to 11.0. Ca^{2+} and Mg^{2+} concentrations are low. SO_4^{2-} concentration is usually high, while that of Cl^- is very low. The water of the lakes is often blue-green in colour due to the mass development of phytoplankton.

A. fusiformis is present as nearly monospecific populations in the highly saline lakes (Iltis 1969a), only a few other phototrophs being found. In contrast, in the mesohaline lakes, *Arthrospira* co-exists with a number of other phototrophs, including other cyanobacteria, diatoms and dinoflagellates. Among the cyanobacteria, *Anabaenopsis arnoldii* and many *Oscillatoria* spp. are often important. Several *Spirulina* species may be present, especially *S. laxissima*, *S. major* and *S. subsalsa*. Studies by Margheri et al. (1975) of two African natron lakes, Lake Mombolo and Lake Rombou, in the region of Kanem north-east of Lake Chad, found these *Spirulina* species as well as several *Arthrospira* spp. Within the phototroph populations of both permanent and temporary lakes, the rotifer density can be very high, although varying seasonally, reaching several hundred individuals per litre (Iltis and Riou-Duwat 1971). In some lakes *Arthrospira* constitutes an excellent feed for the cichlid fish *Tilapia* (Coe 1966; Bergman et al. 2003). Another consumer of *Arthrospira* and other large filamentous cyanobacteria such as *Anabaenopsis* is the flamingo *Phoenicopterus minor*. The pink colour of these birds comes from the carotenoid pigments originating in cyanobacteria (Fox 1976).

The detailed studies by Iltis established a direct correlation between the biomass density of *Arthrospira* and the salinity of the water. The mass blooms occur at salinity values from

Table 25.3 Geographical distribution of the main populations of *Arthrospira*

Country	Location	Taxon	References
AFRICA			
Chad	Natron lakes (Bodou, Mombolo, Rombou, Yoan) and pools (Latir, Iseirom, Latir, Liva), Kanem region	*A. platensis, A. platensis* f. *minor*	Léonard and Compère (1967), Iltis (1972), and Rich (1931)
	Lake Kailala, Lake Kossorom	*A. fusiformis*	Sili et al. (1999)
Kenya	Natron lakes (Bogoria, Crater, Elmenteita, Nakuru)	*A. platensis*	Rich (1931) and Vareschi (1982)
	Rift Valley	*A. fusiformis*	Ballot et al. (2004)
	Lake Bogoria	*A. platensis, A. platensis* f. *minor*	Tuite (1981)
		A. fusiformis	Hindák (1985)
	Lake Simbi	*A. platensis*	Melack (1979)
		A. platensis = *A. fusiformis*	Kebede and Ahlgren (1996)
		A. fusiformis	Ballot et al. (2005)
	Lake Sonachi	*A. fusiformis*	Ballot et al. (2005)
	Lake Oloidien	*A. fusiformis*	Ballot et al. (2009)
Ethiopia	Lake Aranguadi	*A. platensis*	Melack (1979)
	Lake Chiltu, Green lake	*A. platensis* = *A. fusiformis*	Kebede and Ahlgren (1996)
Egypt	Lake Maryut	*A. platensis*	El-Bestawy et al. (1996)
Sudan	Lake Dariba, Jebel Marra	*A. geitleri*	Fott and Karim (1973)
Algeria	Pond Tamanrasset	*A. platensis*	Fox (1996)
Congo	Laka Mougounga	*Arthrospira* sp.	Fox (1996)
	Lake Kivu	*Arthrospira* sp.	Fox (1996)
Zambia	Lake Bangweolou	*Arthrospira* sp.	Fox (1996)
Tunisia	Lake Korba	*Arthrospira* sp.	Fathi et al. (2001)
Mozanbique	Wastewater ponds	*A. fusiformis*	Mussagy et al. (2006)
South Africa	Lake Tswaing	*A. fusiformis*	Oberholster et al. (2009)
ASIA			
India	Ponds	*A. maxima*	Desikachary and Jeeji Bai (1996)
	Lonar Lake; ponds; tank (Madurai, MCRC isolate)	*A. indica*	Desikachary and Jeeji Bai (1992)
Myanmar	Crater lake (Chapter 26)	*Arthrospira* sp.	Min Thein (1993)
Pakistan	Fish pond, Lahore	*Arthrospira* sp.	Fox (1996)
Sri Lanka	Lake Beria	*Arthrospira* sp.	Fox (1996)
China	Fish ponds, Nanking	*A. platensis*	Tsen and Chang (1990)
	Lake Bayannur	*A. platensis*	Zheng et al. (1992)
Thailand	Tapioca factory effluent lakes, Bangkok	*Arthrospira* sp.	Fox (1996)
Russia	Tunatan lake, Siberian steppe	*A. fusiformis*	Voronichin (1934)
Azerbaijan	Water basin, Khumbasha	*Arthrospira* sp.	Fox (1996)
Pakistan	Pond, Lahore	*Arthrospira* sp.	Fox (1996)
AMERICA			
Mexico	Lake Texcoco solar evaporator	*A. maxima*	Busson (1971)
Brazil	Mangueira Lagoon	*Arthrospira* sp.	Greque de Morais et al. (2008)
California	Pond, Oakland	*A. maxima*	Gardner (1917)
	Coastal lagoon, Del Mar	*A. platensis*[a]	Lewin (1980)
Peru	Lake Huachachina	*A. platensis*	Busson (1971)
		A. maxima	Desikachary and Jeeji Bai (1992, 1996)
Uruguay	Montevideo	*A. platensis*[b]	Gomont (1892)
EUROPE			
Spain	Lake Santa Olalla	*Arthrospira* sp.	Rippka and Herdman (1992)
France	Tiny lake, Camargue	*Arthrospira* sp.	Fox (1996)
Hungary	Adasztevel-Oroshaz	*Arthrospira* sp.	Busson (1971)
Romania	Alkaline pond near Cluj-Napoca	*A. fusiformis*	Aldea et al. (2002)
Serbia	Salty puddles near river Tamiš	*A. fusiformis*	Fuzinato et al. (2010)

[a]Reference strain PCC 7345
[b]Holotype

22 to 60 g L^{-1}, high carbonate-bicarbonate concentrations (pH 8.5–11.0) and temperatures from 25°C to 40°C. Generally, the waters populated by *Arthrospira* have a mean salinity of 37 g L^{-1}. However, *Arthrospira* has been found at salinity levels ranging from 8.5 to 200 g L^{-1} and in at least one case up to 270 g L^{-1} (Iltis 1968). Biomass density (as dry weight) can exceed 1 g L^{-1}. Sili et al. (1999) studied two soda lakes (L. Kailala, L. Kossorom) on the north-east fringe of Lake Chad, where a number of small bays and inlets between the old sand dunes are located. Surveys showed that *A. fusiformis* was clearly the dominant (Fig. 25.4), with some filaments of *Anabaenopsis* spp., *Spirulina laxissima* and *Synecochoccus* sp., *A. fusiformis* is also found in some lakes of the northern Algerian Sahara having saline waters (1–8%) and pH levels from 7 to 9. These waters, which have lower alkalinity (about 20 meq L^{-1} of HCO_3^{-1} and CO_3^{-2}), but Na^+ levels similar to those of the highly alkaline saline waters of the East African Rift Valley, may be considered to be sulphate-chloride rich waters (with Cl^- the major anion).

Over the past three decades the impact of excess water abstraction and lack of care with pollution has had a marked effect on some of the Rift Valley lakes, where *Arthrospira* commonly is found, with a marked deterioration in their ecological status and a progressive increase in nutrient concentration. The two lakes Naivasha an Oloiden (Kenia) are typical examples of this recent degradation and salinization which has promoted the appearance of *Arthrospira* (Ballot et al. 2009). The Lake Oloidien hypereutrophic alkaline modification of the originally freshwater has caused a shift in species dominance from coccoid Chlorophyceae to *A. fusiformis* and *Anabaenopsis elenkinii*. The main sources of nutrient input are cattle and goat herds, watering at the lake and local women washing clothes using detergents (Ballot et al. 2009).

In Lake Nakuru, the dominance of *A. fusiformis* has been documented since the 1960s (Talling and Talling 1965). Okoth et al. (2009) demonstrated that *A. fusiformis* was the most dominant species in terms of population density in Lake Nakuru. However, the pollution effects due to the urbanization process near the lake is expected to influence the lake's phytoplankton community strongly in the future.

As previously mentioned, a massive population of *A. maxima* was found in some of the external sectors of the gigantic spiral-shaped solar evaporator used to extract salts from the saline carbonate-bicarbonate rich waters of Lake Texcoco, Mexico. The evaporator lies 2,200 m above sea level and is about 1 m deep. The alkaline (pH 10) saline water is moved slowly from the outer part of the evaporator towards the centre (Gallegos 1993). The water is high in HCO_3^- and CO_3^{2-}, Cl^- and Na^+ and the total salt concentration ranges from 11 to 39 g L^{-1} (Busson 1971). In the past the company that operated the plant, Sosa Texcoco (and later Spirulina Mexicana), had its "Spirulina" (*A. maxima*) production facilities in the outside rim of the solar evaporator, where it was present in almost monospecific populations, being associated with only a few other cyanobacteria such as *Synechocystis aquatilis*. In 1994 Sosa Texoco ceased cultivation operations of *Arthrospira maxima* in the external ponds of the "El Caracol" solar evaporator (Alcocer and Williams 1996). At present Lake Texococo is reduced to several separate waterbodies (Caracol, Lago recreativo and Nabor Carillo) from where a number of *A. maxima* strains have been isolated (Dadheech et al. 2010).

More recently, *Arthrospira* has been found occasionally in domestic wastewater treatment plants. in Maputu, Monzambique (Mussagy et al. 2006) and Turkey (G. Torzillo, unpublished data), both characterised by pH ranging between 7.1 and 7.7 and a conductivity between 1,000 and 2,000 μS cm^{-1}. In contrast to what has sometimes been believed, this suggests that *Arthrospira* can acclimate to new environments not markedly or exclusively alkaline.

25.5 Physiology

The aim of this section is to point out relevant areas in which *Arthrospira* has been used as a model organism, together with other studies with data of significant importance in understanding its growth, physiology and biochemistry, especially when grown outdoors. One of the basic principles of microalgal technology is to re-create the environmental and cultivation conditions permitting high productivity in a monoalgal culture. Without doubt light and temperature are the most important. In nature, the organism uses its gas vacuoles to regulate its position within the underwater light gradient and follow daily and seasonal changes in light flux. In shallow culture ponds, however, light availability has to be optimized by modifying operational parameters such as optimal cell density and the degree of mixing and culture depth in order to obtain the highest productivity (Vonshak et al. 1982; Richmond 2004b; Belay 2008).

Adequate mixing coupled with maintenance of high cell densities can to a certain extent reduce the effect of light saturation of photosynthesis and damage to the photosynthetic apparatus due to prolonged exposure to excess of light (photoinhibition) (Torzillo and Vonshak 2003). Sufficient mixing also ensures an even distribution of nutrients and oxygen degassing of the culture, and at the same time prevents filament aggregation and sinking (Torzillo et al. 2003a).The fact that *Arthrospira* requires high alkalinity for growth ensures selective conditions in the growth medium and *Arthrospira* grown in outdoor open ponds remains a quasi-monoculture (Vonshak et al. 1983). Moreover, when other essential growth nutrients are present in sufficient concentrations, the elevated concentration of bicarbonate-carbonate favours the high productivity observed in nature.

25.5.1 Response to Environmental Factors

25.5.1.1 Effect of Light on Growth

Zarrouk (1966) was the first to study the response of *Arthrospira maxima* to light and concluded that growth rate reached a maximum when cultures were grown under a light irradiance between 25 and 30 klux (340–400 µmol photon m^{-2} s^{-1}). However, the lack of information about the strain used, on how light was measured and its path in the culture vessel, makes the results difficult to compare with later studies. Data gathered in our laboratory (Institute of Ecosystem Study, Firenze, Italy) with *A. fusiformis* strain M2, which was originally isolated from Mombolo Lake (Chad) (Balloni et al. 1980), showed that growth becomes light-saturated in the range of 150–200 µmol photon m^{-2} s^{-1} (Bocci et al. 1980); this value for irradiance is about one order of magnitude less than recorded outdoors in summer days (1,850–2,000 µmol photon m^{-2} s^{-1}). By using a turbidostatic culture, a maximum growth rate of 0.063 h^{-1} was estimated for *A. maxima* strain 4 Mx, isolated from Texoco Lake, Mexico, and 0.059 h^{-1} for *A. fusiformis* strain K4, isolated Rombou Lake, Chad (Balloni et al. 1980), which correspond to doubling times of 11 and 11.7 h, and a light transformation efficiency of 7.2% and 7.8% of the photosynthetically active radiation (PAR, 400–700 nm) for *A. maxima* and *A. fusiformis*, respectively (Bocci et al. 1980). Based on a requirement of 20 quanta mol^{-1} CO_2, Ogawa and Aiba (1978) estimated the slightly higher value for photosynthetic efficiency of 11.4% PAR.

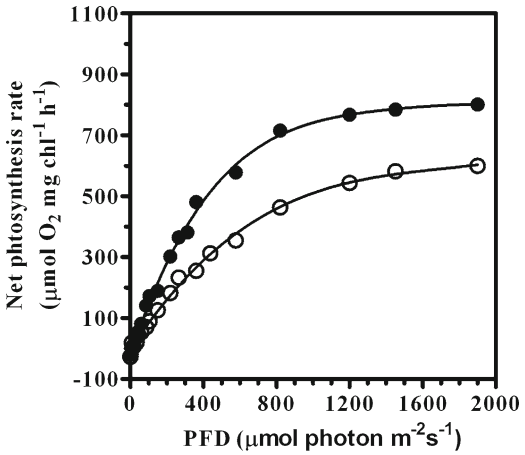

Fig. 25.13 Photosynthetic light response curves of *A. fusiformis* strain M2 (○ photoinhibition; ● control) for 45 min at a PFD of 2,500 µmol photon m^{-2} s^{-1}

Table 25.4 Photosynthesis-irradiance (P/I) parameters estimated from *Arthrospira fusiformis* strain M2 cultures before and after 45 min photoinhibition at a PFD of 2,500 µmol photon m^{-2} s^{-1} at 35°C

Parameter	Unit	Control	Photoinhibited
P_{max}	µmol O_2 mg chl^{-1} h^{-1}	800	600
I_K	µmol photon m^{-2} s^{-1}	430	610
α	µmol O_2 (mg chl µmol photon m^{-2} $s^{-1})^{-1}$ h^{-1}	1.915	1.094
R	µmol O_2 mg chl^{-1} h^{-1}	–20.5	–26.5

P_{max} light saturated rate, I_k saturation irradiance, α initial slope of the P/I curve, R dark respiration rate

25.5.1.2 Light Stress – Photoinhibition

Different strains of *Arthrospira* may differ in their sensitivity to light stress (Vonshak et al. 1988b; Vonshak and Novoplansky 2008). Photoinhibition, which is the loss of photosynthetic capacity due to damage to photosystem II (PSII) caused by photon flux densities (PFD) in excess of those required to saturate photosynthesis, can be manifested in various ways. These include a decrease in the maximum quantum yield of CO_2 uptake or O_2 evolution, a decrease in the convexity of the photosynthesis light response curve (P/I), and, in the case of prolonged exposure to excessive light, a decrease in the light-saturated photosynthesis (Leverenz et al. 1990; Long et al. 1994). In at least one *A. fusiformis*, strain P4P, photoinhibition was probably due to a change in the turnover rate of a specific protein, D1, which is part of PSII. Observed differences may be genotypic or environmental responses. Cultures acclimated to high light intensities exhibit a higher resistance to photoinhibition (Vonshak et al. 1996a). In a study of the photosynthetic light response curve of *A. fusiformis* strain M2 cells exposed to strong light (Torzillo and Vonshak 1994), photoinhibition caused a 48% reduction in the initial slope of the P/I curve compared to the control (Fig. 25.13). However, there was less effect on P_{max} (maximum saturation rate), which was reduced by about 25% (Table 25.4). These results emphasize that photoinhibition not only affects $P_{max,}$ but actually has a stronger effect on the initial slope of the P/I curve i.e. on light-limited photosynthetic activity. In contrast to higher plants and microalgae, in the case of cyanobacteria it has always assumed do not use an antenna-related quenching mechanism to decrease the amount of energy funneled to photosystem II (PSII) reaction centre (Campbell et al. 1998). Recently, however, evidence has been presented for the existence of at least three distinct mechanisms for dissipating excess energy in cyanobacteria. One of these photoprotective mechanism is related to the phycobilisomes, the extramembranal antenna of cyanobacteria PSII. In this photoprotective mechanism the orange carotenoid protein (OCP) in *Synechocystis* sp PCC 6803 plays an important role (Kirilowsky 2007; Wilson et al. 2006). The *Arthrospira maxima* OCP contains 3'-hydroxyechinenone (Kerfeld 2004). However, mechanism of this novel photoprotective process in cyanobacteria awaits elucidation.

25.5.1.3 Effect of Temperature on Photosynthesis and Respiration

A detailed study of the response of an *A. fusiformis* strain M2, to temperature was performed by Torzillo and Vonshak (1994). They determined the optimal temperature for photosynthesis, and also measured the effect of temperature on the

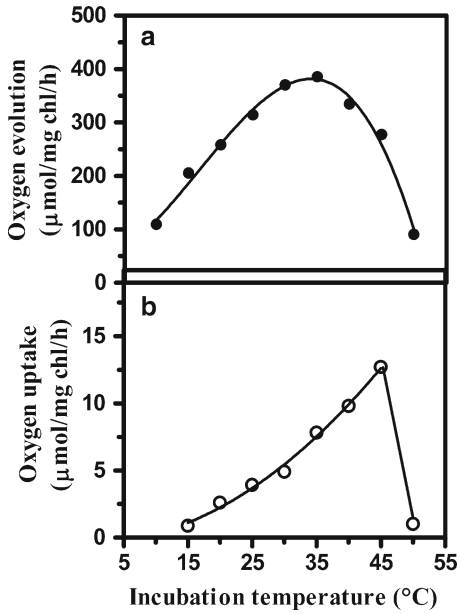

Fig. 25.14 Effect of temperature on photosynthesis (**a**), and dark respiration (**b**) rates of *A. fusiformis* strain M2. Cells were allowed to equilibrate for 15 min at each temperature before measurement

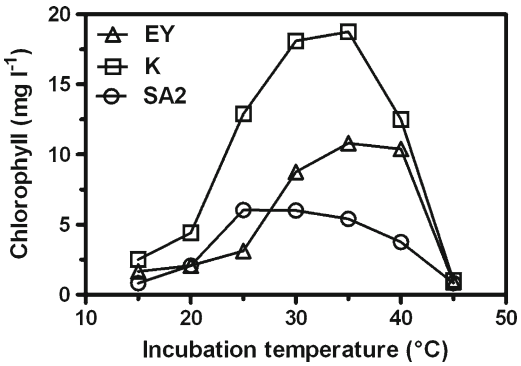

Fig. 25.15 Temperature responses of three *Arthrospira fusiformis* isolates measured as the increase in chlorophyll concentration after incubating the cells for 4 days on a temperature block under constant light (100 µmol photon m^{-2} s^{-1})

dark respiration rate by following the O_2 uptake rate in the dark. The optimal temperature for photosynthesis was 35°C (Fig. 25.14a), while dark respiration was highest at 45°C (Fig. 25.14b). At temperature of 50°C the respiration rate dropped to almost zero. At temperature extremes outside those optimal for growth, both respiratory and photosynthetic activity declined. However, the sensitivity of respiration to such extremes was significantly greater than the sensitivity of photosynthesis under the same conditions. Under conditions where respiration was completely inhibited, photosynthetic oxygen evolution was maintained at about 30% of the optimum value. A temperature-dependent exponential relationship between 15°C and 45°C was obtained, with the respiration rate increasing as temperature increased. The temperature-dependent dark respiration rate is given by:

$$R = 0.771.e^{(0.0636.T)}$$

where R is the respiration rate (µmol O_2 mg^{-1} chl h^{-1}) and T is the temperature (°C). An Arrhenius plot for respiration showed an activation energy of 48.8 kJ mol^{-1} for *Arthrospira*. The temperature coefficient (Q_{10}) calculated for the 15–45°C range was 1.85. The respiration-to-photosynthesis ratio in *Arthrospira* was 1% at 20°C and 4.6% at 45°C (Torzillo and Vonshak 1994). These low values confirmed the general assumption that cyanobacteria have low respiration rates (van Liere and Mur 1979). However, the respiration-to-photosynthesis ratios measured in these experiments were found to be much lower than those reported for outdoor cultures of *Arthrospira*, where up to 34% of the biomass produced during the daylight period may be lost through respiration at night (Guterman et al. 1989). Torzillo et al. (1991) concluded that night biomass loss due to dark respiration depended on the temperature and light irradiance to which the cultures were exposed during the day, and that this occurred because of the strong effect of temperature and light on cell composition. Analysis of the biomass harvested at sunset and sunrise revealed that the protein content was ca. 57% and 71%, of dry weight respectively, whereas the carbohydrate content during the same periods was ca. 34% and 19%, of dry weight respectively. An increase in carbohydrate in cells was observed when these were grown at the suboptimal temperature of 25°C. Excess carbohydrate synthesized during the day was then partially used for protein synthesis at night, while most of them were lost by respiration (Torzillo et al. 1991).

25.5.1.4 Effect of Temperature on Growth and Cell Composition

In nature *Arthrospira* is found in permanent or temporary water bodies at relatively high temperatures. The optimal temperature for laboratory cultivation of this organism is about 35°C. However, many *Arthrospira* strains differ in their optimal growth temperature as well as in their sensitivity to extreme values. For instance, in the comparison of strains shown in Fig. 25.15, strain SA2 has a relatively low optimal growth temperature (24–28°C), while the strain EY grew well up to 38–40°C. The isolate marked K was characterized by a relatively wide range of optimal growth temperatures: 28–36°C. This is just one example of the variations that exist among the different strains of *Arthrospira*, as regards optimal growth temperatures. Significant differences in the maximum temperature for growth have been also found within the two species of *A. maxima* and *A. fusiformis*. *A. maxima* strains were apparently unable to grow above 36°C, while some *A. fusiformis* strains were found to be capable of growing at a temperatures higher than 40°C (Tomaselli et al. 1987). *Arthrospira fusiformis* strain M2 was

able to grow up to 42°C and to withstand 46°C for several hours. This strain has also been grown successfully in closed photobioreactors (Torzillo et al. 1986). However, when *A. fusiformis* strain M2 was grown under light-limited turbidostatic conditions at its maximum tolerable growth temperature (42°C), there was a marked decrease in photosynthetic pigment and protein levels. Under these conditions, an increase in carbohydrate cell content associated with changes in the degree of fatty acid saturation (i.e. reduction in γ-linolenic acid synthesis in favour of linoleic acid accumulation) was also observed (Tomaselli et al. 1988). It has been shown that *Arthrospira* is able to accumulate glycogen, under high light and nitrogen limitation, therefore it has been proposed as a potential feedstock for fermentative production of biofuels (Aikawa et al. 2012).

25.5.1.5 Interaction of Low Temperature with Light and High Oxygen Concentration

It is suggested that *Arthrospira* cultures grown at suboptimal temperatures are more sensitive to photoinhibition than those grown at the optimal value. The latter will be able to better handle excessive light energy, since they have a higher rate of electron transport, an active repair mechanism and more efficient means of energy dissipation (Vonshak 1997b). The saturation pulse method was applied in order to study the synergistic effect of high oxygen concentration and low temperature in outdoor cultures of *A. fusiformis* strain M2 (Torzillo et al. 1998). It was observed that the combination of low temperature (25°C) and high oxygen concentration (70–80 mg L^{-1}) had considerable impact of PSII photoinhibition evaluated as changes in the F_v/F_m ratio (variable to maximum fluorescence). The F_v/F_m ratio decreased down to 64% of the morning value in the middle of day in the high oxygen culture (Fig. 25.16). When high oxygen concentration was combined with low temperature, the F_v/F_m ratio decreased to up to 45% of the morning value. Complete recovery of the F_v/F_m ratio occurred in the afternoon only in the low oxygen cultures, while it reached about 80% of the morning value in the high oxygen culture, and only 63% in the culture exposed to combination of high oxygen and low temperature (Fig. 25.15). Photoinhibition studies in the cyanobacterium *Synechocystis* sp. PCC 6803 have indicated that photodamage is initiated by the direct effect of light on the oxygen evolving complex (OEC) and that reactive oxygen species (ROS) inhibit the repair of photodamaged photosystem II primarily suppressing the synthesis of proteins *de novo* (Murata et al. 2007).

The photosynthetic electron transport system represents the major source of ROS, that is, singlet oxygen, hydrogen peroxide and the superoxide radical (Asada 1994; Krause 1994). When scavenging of potentially damaging oxygen species is insufficient, photoinhibition can occur. High oxygen concentration itself can influence the growth and the photosynthetic activity of the cultures even when the light intensity is relatively low, as demonstrated by several experiments carried out both in the laboratory and outdoors (Torzillo et al. 1984; Marquez et al. 1995; Vonshak et al. 1996a). The combination of high oxygen concentration and high light intensity can be a very common event in outdoor cultures, particularly in closed systems. For example, in well growing *A. fusiformis* strain M2 cultures in tubular photobioreactors (i.d. 5 cm), the oxygen concentration can increase at a rate of 2–3 mg L^{-1} min^{-1}. This results in an oxygen concentration of up to 70–80 mg L^{-1} (Torzillo et al. 1998).

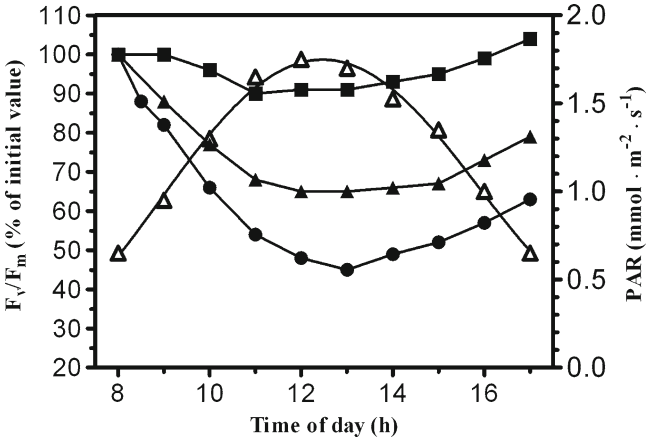

Fig. 25.16 Effect of oxygen concentration and temperature on maximum quantum yield of PSII (F_v/F_m) of *Arthrospira fusiformis* strain M2 cultures grown outdoors in photobioreactors. (■) Low oxygen- optimal temperature; (▲) high oxygen – optimal temperature; (●) high oxygen-low temperature; (Δ) PFD

Sensitivity to stress is strongly influenced by the light acclimation conditions of the cells. Cells acclimated at low light usually saturate photosynthesis at lower light intensities (Ramus 1981). Measurements of chlorophyll fluorescence quenching in outdoor cultures of *Arthrospira* showed that sensitivity to photoinhibition strongly increased in *A. fusiformis* strain M2 cultures acclimated to low light. Yet, despite a 40% reduction in the F_v/F_m ratio observed in cells exposed to high stress conditions i.e. a combination of suboptimal temperature (25°C) and high dissolved oxygen concentrations (up to 60 mg L^{-1}), no significant loss in the D1 content was found. By contrast, under the same stress conditions, the D1 content was reduced by 50% in low-light acclimated cultures (Torzillo et al. 2003a). Productivity of low-light acclimated cultures was lower than that obtained in high-light acclimated cultures. Light acclimation also strongly influenced the effect of stress on the pigment composition of the *A. fusiformis* strain M2 cells. Pigment analysis showed that ß-carotene decreased during the day in high stress cultures, while the mixoxanthophyll and zeaxanthin content increased. The considerable presence of mixoxanthophyll and zeaxanthin may provide a source of defense for cells against photooxidation (Torzillo et al. 2003a).

25.5.2 Effect of Salinity on Growth, Photosynthesis and Respiration

The occurrence of *Arthrospira* spp. in marine habitats has not been documented, at least not as dominant blooms like those found in the natron lakes. This is most likely due more to the very low content of bicarbonate rather than to the high content of NaCl. *Arthrospira*, unlike marine organisms, does not require high NaCl concentration for growth; rather, it only tolerates the salt. The exposure of *Arthrospira* cultures to high NaCl concentrations at first results in an immediate cessation of growth; after a lag period, a new steady state of growth is established (Vonshak et al. 1988a). The length of the time lag is exponentially correlated to the degree of salinity stress imposed on the cells. In many cases, this lag is associated with a decline in chlorophyll and biomass concentrations in the culture (Vonshak et al. 1988a).

When the biomass composition of two strains grown under different salt stress conditions was compared, significant changes, mainly an increase in carbohydrate and a decrease in the protein levels, were observed. These changes correlated with the degree of stress imposed (i.e. higher level of carbohydrate at a higher salt concentration). The difference in the level of carbohydrate accumulated by the two strains may also reflect a difference in their ability to acclimate to salt stress. Changes in lipid synthesis have also been demonstrated: in salt-stressed cells, with an increase in lipid content and in the degree of fatty acid saturation is observed with specific modifications in the long-chain fatty acids (C18). In *A. fusiformis* strain M2 the oleic acid content doubled when the organism was grown in presence of 0.5 M NaCl (Tomaselli et al. 1993).

It has been suggested that exposure to high salinity is accompanied by a higher demand for maintenance energy by the stressed cells (Blumwald and Tel-Or 1982). Changes in the photosynthetic and respiratory activities of an *Arthrospira* strain were measured over a period of 48 h beginning 30 min after exposure to 0.5 and 1.0 M NaCl. A marked decrease in the photosynthetic oxygen evolution rate was observed 30 min after exposure to the salt. This decline was followed by a recovery period, characterized by a lower steady state rate of photosynthesis (Vonshak et al. 1988a). The immediate inhibition of the photosynthetic and respiratory systems after exposure to salt stress has been explained by Ehrenfeld and Cousin (1984) and Reed et al. (1985), in, respectively, *Dunaliella* and in *Synechocystis*. A short-term increase in cellular sodium concentration was due to a transient increase in the permeability of the plasma membrane during the first seconds of exposure to high salt concentration. It has been suggested that the inhibition of photosynthesis due to the rapid entry of sodium, might be the result of the detachment of phycobilisomes from the thylakoid membranes (Blumwald et al. 1984).

High rates of dark respiration in cyanobacteria due to salinity stress had been reported previously (Vonshak and Richmond 1981; Fry et al. 1986; Molitor et al. 1986). This high activity may be associated with the increased level of maintenance energy required for pumping out the excess of sodium ions. Variable chlorophyll fluorescence was applied by Lu et al. (1999) in order to study the kinetics of the response of *A. fusiformis* strain M2 cells to salinity stress. The study revealed that the response of PSII photochemistry during the first 12 h of their exposure to high salinity consisted of two phases. The first phase, which took place during the first 4 h, was characterized by an immediate drop in F_v/F_m during the first 15 min (about 26%) after exposure. This was followed by a recovery to around 92% of the original level after 2 h incubation. After 4 h from the beginning of the salt treatment, another decline in F_v/F_m was observed, which reached about 70% of the initial value after 12 h (Lu et al. 1999). According to Lu and Vonshak (2002), salt stress in *A. fusiformis* strain M2 inhibited the electron transport at both the donor and acceptor sides of PSII, and resulted in damage to the phycobilisomes and a partial disconnection of these from PSII which shifted the distribution of excitation energy in favour of the PSI (state 2). An alteration in the structure of the phycobilisomes of *A. fusiformis* in response to an enhanced Na^+ level was also reported by Verma and Mohanty (2000). The effect of salt stress on *A. fusiformis* was further investigated by Gong et al. (2008). The results confirmed previous observations that salt stress leads to damage on the reducing side of PSII and, in particular, to a modification of the Q_B niche. Moreover, the authors confirmed that also the donor side of PSII was affected by salt stress, and that this damage was associated with a dissociation of the PsbO protein from the thylakoid membranes (Gong et al. 2008).

Another modification in salt-stressed cells is an increase in the respiration rate (Vonshak et al. 1988a). In *A. fusiformis* strain M2, this increase was two- and three-fold when the concentration of salt in the medium was increased from 0.5 to 0.75 mM, respectively (Zeng and Vonshak 1998). Sensitivity to photoinhibition was found to be enhanced when *A. fusiformis* strain M2 cells were exposed to sizable combinations of high light and salt stress (Zeng and Vonshak 1998). It seems that many cyanobacteria are capable of compensating the reduction in the energy supply coming from the photosynthetic pathway by significantly increasing their respiratory activity, which in addition can provide carbon skeletons for the synthesis of organic osmolytes and for the extrusion of Na^+ in cells in order to maintain the osmotic balance (Peschek et al. 1994).

25.5.3 Osmoregulation

During the course of acclimation to salinity, an osmotic adjustment is required (Borowitzka 1986). In *Arthrospira*

this involves the accumulation of low molecular weight carbohydrates. These have been identified as a nine carbon heteroside called glucosyl-glycerol, plus a trehalose (Martel et al. 1992). Changes in the amount of cellular trehalose and in the activity of the enzyme malthooligosyl trehalose hydrolase (Mth), the enzyme that catalyses the formation of trehalose from maltooligosyl trehalose, were recently investigated in *A. fusiformis* by Ohmori et al. (2009). These authors observed that the amount of trehalose in the cells increased rapidly when a high concentration of NaCl was added to the culture medium. The inhibition of sodium ion transport by means of amiloride, a sodium channel blocker, and monensin, a cation ionophore, significantly decreased the amount of cellular trehalose, suggesting that the influx of Na^+ into the cells was connected with an accumulation of trehalose. The synthesis of trehalose was comparable when KCl was used to replace NaCl, while the effect on cellular trehalose synthesis was only half as much by the same amount of LiCl. It was suggested that the cells accumulated trehalose mainly via ionic stress, rather than by osmotic stress. It was also suggested that the influx of Na^+, and not the mere presence of Na^+ in the medium, is connected with the physiological effect of NaCl. It was concluded that the addition of NaCl activated Mth and, at the same time, triggered a transcription of the mth gene. Disruption of the mth gene, if such is possible, may provide further evidence on the role of Mth in the stress-response accumulation of trehalose in *Arthrospira*.

25.5.4 *Arthrospira* as an Alkaliphile

Most *Arthrospira* species currently grown in mass culture were isolated from alkaline and saline or brackish waters characterized by high levels of carbonate-bicarbonate and high pH levels (Sect. 25.4). The physiological characteristics which enable a microorganism to thrive in such an extreme environment are still not fully understood. *Arthrospira* not only survives at high pH values, but actually thrives in such conditions. Belkin and Boussiba (1991), who compared the growth pH optima for the two cyanobacteria *Anabaena* and *Arthrospira*, demonstrated that while the optimal pH for *Anabaena* was in the range of 6.8–7.2, the maximal growth rate for *Arthrospira* was obtained in the 9.5–9.8 range. When incubated at pH 7.0, the growth rate of *Arthrospira* was severely inhibited and was only 20% of that under the optimal conditions. This high pH (>8) requirement clearly defines *Arthrospira* as an obligatory alkaliphile (Grant et al. 1990).

One of the major problems faced by cells in a highly alkaline environment is that of regulating their internal pH. Belkin and Boussiba (1991) were the first to measure the ability of *Arthrospira* to maintain a pH gradient across its cytoplasmic membrane. It was shown that, at external pH values of 10.0 and 11.5, the intracellular pH values were only 8.0 and 8.5, respectively.

Many alkaliphiles require sodium in order to survive in extreme alkaline environments (Horikoshi and Akiba 1982). Padan et al. (1981) demonstrated that an active sodium – proton antiporter is required in order to maintain a low internal pH. Most cyanobacteria require sodium in an mM or submillimolar concentration range in order to maintain viability, especially at an alkaline pH (Miller et al. 1984; Espie et al. 1988). Some reports have indicated, in cyanobacteria incubated in a sodium-deficient media, the loss of the photosynthetic oxygen evolution (Zhao and Briand 1988) and a degradation of the thylakoid membranes (Averdano et al. 1989). The effect of sodium deprivation is very dramatic in *A. fusiformis*. In this obligate alkaliphilic cyanobacterium, a strict Na dependence has been found for both the growth and photosynthetic activity of the cells (Schlesinger et al. 1996). It has been demonstrated that concentrations of Na^+ no less than 50 mM in the medium are required in order to maintain growth at a high pH. Depletion of Na^+ causes a light-dependent inhibition of PSII and a loss in the phycocyanin content of *A. fusiformis* cells (Schlesinger et al. 1996). It has been suggested that pigment bleaching and a loss of PSII activity are due to the inability on the part of the cells to maintain an appropriate intracellular pH. Pogoryelov et al. (2003) theorized that the specific site of inactivation of the photosynthetic electron transport at alkaline pH lies in the water-splitting enzyme (oxygen evolving complex) and, in contrast with earlier reports, that the inactivation occurs in the dark and for short periods, without any detectable damage to the photosynthetic chain. A very intriguing question is how *Arthrospira* is capable of coping simultaneously with two extreme environmental situations: a high pH and a high concentration of Na^+. In fact, not only does the adaptation of cells in this organism permit growth at high pH and high Na^+ concentrations, as in the case of many other alkali-tolerant cyanobacteria, but also the cell functions are optimized to these extreme conditions (pH 9.5–11.5 and 150–250 mM NaCl). Further studies on the energy requirement and involvement of light in the regulatory process are needed.

25.5.5 How Does *Arthrospira* Compete in Culture?

Studies performed on different *Arthrospira* isolates from various sources demonstrate highly variable responses to different ecological factors (Tomaselli et al. 1987; Vonshak et al. 1996a, b). It is on the basis of this variability that strains suitable for the biotechnological applications are selected: i.e. photo-, thermo- or osmotolerant strains (Tomaselli et al. 1993; Vonshak and Guy 1992; Vonshak et al. 1988b). Detailed studies aimed at measuring the ecological specificity

of the different species, particularly of those utilized for commercial purposes, are scarce (Kebede and Ahlgren 1996). Nevertheless, full knowledge of the ecological demands of the chosen species and the use of more productive selected strains is important if high productivity is to be achieved in intensive outdoor cultivation systems. The use by commercial producers of multistrain inocula which respond positively to changes in abiotic factors (due to their heterogeneity) represents another way of compensating for the present lack of well-characterized strains.

Like other components of the phytoplankton, *Arthrospira* plays a role in cycling nutritional elements and in their passage into different food chains; moreover, it provides an important direct food source for zooplankton, fishes, birds and humans. The same factors that in the original habitat regulate the occurrence of *Arthrospira* in monospecific populations are those required for both successfully maintaining a monoculture in intensive production ponds outdoors and for achieving high productivity. Factors such as high alkalinity and salinity are the key features that selectively limit the growth of other organisms and predators. Moreover, once the optimal conditions for the growth of *Arthrospira* are maintained in ponds, this organism successfully outcompetes other occasionally occurring phototrophs for nutrient and light. This may be attributable to its fast growth rate and photoacclimation capability, and its high tolerance to environmental stress (irradiance, salinity, temperature).

25.6 Mass Cultivation of *Arthrospira*

At present *Arthrospira* (commercially indicated as Spirulina) represents the second most important commercial microalga in term of US$ (after *Chlorella*), while in terms of total biomass produced, the *Arthrospira* market is twice or more of that occupied by *Chlorella* (Torzillo and Vonshak 2003). Commercial production takes two broad approaches: that of industrialised countries who interested in producing the biomass for health food market, as well as for the extraction of fine chemicals, and that of developing countries looking for a rich source of protein which can be produced locally, using marginal land and saline water unsuitable agriculture, and the opportunity for treating animal and human waste (Laliberté et al. 1997; Habib et al. 2008). A major producer of *Arthrospira* is the DIC group of companies, Earthrise in California, USA, Hainan DIC Marketing in Hainan Island, China. These facilities produce about 1,000 t *Arthrospira* annually (Belay 2008). Another important *Arthrospira* producer is Cyanotech Corporation of Hawaii with an annual production of 300 t (dw). Other producers are located mainly in the Asia-Pacific region, particularly China and India (Lee 1997) and the highest production capacity of *Arthrospira* biomass takes place in China. Recent estimates are that the total potential of the different sites in China now exceeds 2,000 t (dw), but the situation is changing rapidly due to the development of production on the Ordos Plateau of Inner Mongolia (Lu et al. 2011). Although the average annual temperature in the region is only 6.4°C (Qiao et al. 2001), which poses a challenge (Li et al. 2003), commercial production under greenhouses permits lower costs than elsewhere in China and it is aimed to reach an annual output of 3,000 t within a few years. Lu et al. (2011) state that a 1,000,000-t plan for production has been sketched out.

Arthrospira production is mostly carried out in raceway ponds of 2,000–5,000 m^2 and may contain between 400 and 1,000 m^3 of culture according to the depth adopted, which can vary between 15 and 40 cm depending on season, desired algal density and, to a certain extent, the desired biochemical composition of the final product (Shimamatsu 2004; Belay 2008). Further information on commercial production of *Arthrospira* (Spirulina) the reader can be found in more specialized books (Vonshak 1997b; Richmond 2004a; Gershwin and Belay 2008).

The major share of the market for this organism is for health food involving crude biomass production. This has the advantages of simple processing (harvest and rudimentary handling) keeping production costs reasonably low, and to elude the competition with chemical industry which cannot match the wealth in nutritional bioactive components and attractiveness of natural products (Boussiba and Affalo 2005).

The experience gathered over many years of industrial production of *Arthrospira* has allowed to individuate the main problems related with commercial scale production (Shimamatsu 2004). They are: (1) how to maintain an unialgal production, that is, without sizeable presence of contaminants all over the year, and (2) how to maintain a consistent quality of the product. These two aspects are strongly dependent on an appropriate strain selection and adequate formulation of the culture medium. Major criteria for the selection of strains are growth rate, biochemical composition and resistance to environmental stress, usually high light and low and high temperatures at each production site. The chemical composition of culture medium is similar to than used for laboratory culture (Zarrouk's medium) with a high content of $NaHCO_3$ (16.8 $g\,L^{-1}$), K_2HPO_4 (0.5 $g\,L^{-1}$) and KNO_3 (2.5 $g\,L^{-1}$), pH is maintained at 9.5–10.3 by artificial CO_2 supply. The high alkalinity and high pH represent an efficient barrier against contamination by other microalgae, permitting the maintenance of a unialgal culture in open ponds. The cost of nutrients usually dictates the choice of nutrient to be used. For example, the use of ammonia, which is cheaper than nitrate, is restricted by the fact that it can be toxic to the cultures. The predominance of NH_3 over NH_4^+ at pH values greater than 9.25 (the pKa of the ammonia system at 25°C), together to the high permeability of the NH_3, causes uncoupling of photosynthesis in *Arthrospira* cells (Boussiba 1989;

Boussiba and Gibson 1991). At pH values of 9.5 which is considered optimal for the growth of *Arthrospira,* concentration of free ammonia above 2.5 mM is reported to be toxic to many algae (Abeliovich and Azov 1976; G. Torzillo, unpublished data). For this reason, the fed-batch addition of ammonia-based nitrogen sources is mandatory to effectively prevent any inhibiting effect (Rodrigues et al. 2011). Moreover, the fed-back addition of ammonia-nitrogen can also reduce the risk of nitrogen deficiency which may occur when ammonium salts are used as the only source of nitrogen and ammonium is lost by out-gassing. For this reason the simultaneous use of nitrates and ammonium salts are often used *Arthrospira* farms to avoid the risk of nitrogen depletion.

Another important nutrient component of the culture medium is the carbon source. The carbon content of the *Arthrospira* biomass is about 50% (Torzillo et al. 1991), meaning that a minimum of 1.8 g of CO_2 to synthesize 1 g (dry weight) of biomass is required. Therefore, the utilization of CO_2 in the flue gas may help to reduce the cost of the biomass production (Ferreira et al. 2012). Strategies for supplying CO_2 in large-scale cultivation ponds to increase the CO_2 absorptivity have been proposed (Bao et al. 2012).

Cultures are operated according to a semi-continuous regime where a constant cell concentration is maintained in the ponds, usually in the range of 400–600 mg L^{-1} dry weight, by daily harvesting of biomass to the extent that it has grown over the last 24 h. This concentration range is mainly dictated by harvesting efficiency. However, a concentration below 100 mg L^{-1} dry weight can make cells susceptible to photoinhibition (Vonshak and Guy 1992; Torzillo et al. 1998). This phenomenon can occur at the start of the pond inoculation, when the culture is too dilute and not sufficiently acclimated to high light conditions, or when cultures are subject to a combination of high light and low temperature (Sect. 25.5.1).

Maintaining a culture in large open ponds without any contamination by other organisms is one of the major challenge. Vonshak and Richmond (1988) estimated the overall annual loss of productivity due to contamination and subsequent discarding of culture to be in the order of 15–20%. Dilution of the cultures caused by rainfall and low light conditions often facilitates the onset of microalgae (*Chlorella* in particular), protozoa and insects. Continuous recycling of medium can easily result both in the concentration of contaminants like *Chlorella* or *Arthrospira* strains characterized by having short filaments, that are not arrested by the filters, and in the accumulation of organic matter because of decomposition of death algae. This situation usually stimulates the growth of graze particularly protozoa. In order to prevent this situation it is mandatory to maintain the cultures at the optimum growth conditions, by continuous monitoring of the physiological state of the cultures using fast and reliable measurements such as chlorophyll fluorescence technique (Sect. 25.5.1), and to reduce as much as possible the stress damage mechanically created by excessive stirring or by use of centrifuge pumps for culture transfer (e.g. during the harvesting). In most cases, the steps which proved effective in preventing contamination by *Chlorella* were: maintaining a high bicarbonate concentration (e.g. 0.2 M); and taking precautions to maintain the dissolved organic load in the culture medium as low as possible. Amoeba grazing on *Chlorella,* and *Arthrospira* have been also observed in some improperly operated commercial ponds. Addition of ammonia (2 mM) arrested the development of these grazers (Vonshak et al. 1983). Similar observations have been reported by Lincoln et al. (1983), who showed that the population of grazers was significantly reduced when ammonia was used as the main N source. However, ecological control by predators, although this has occasionally been observed in natural lakes dominated by *Arthrospira* (e.g. Lake Ankorongo, Madagascar), has been never applied in industrial cultures (Shimamatsu 2004).

Safety and quality control of biomass represent another important issue for the industrial production of *Arthrospira* (Belay 2008). Toxic species of other cyanobacteria may be present (Chap. 24) and some *Arthrospira* biomass companies have developed methods for their determination and actually certify their products to be free of toxins, such as microcystins. *Arthrospira* biomass does not contain microcystin (An and Carmichael 1996), but contamination by other cyanobacteria cannot be ruled out. A potential problem of open ponds of *Arthrospira* production is that the water may be contaminated with pathogenic organisms. Handling of the product during processing can also result in microbial contamination (Belay 2008). Production cost is an area where little information is available as companies are usually not available to provide this information. However, cost of production is estimated to range from US$ 6–12 kg^{-1} dry matter. The lower figure is based on a "feed" grade product, not for direct human consumption. The product is sun-dried and contains a high ash and low chlorophyll content. The demand for such a product is continually growing, as its relatively low price is making it more attractive to the feed industry as an additive to fish and chicken feed. This demand is reflected in the reports of an increase in the production of feed grade products up to 60 t $year^{-1}$ by Earthrise Farms, compared to only a few tonnes of feed grade produced some years ago. A starch-producing factory in Thailand has started to use its water refuse from an anaerobic digester to grow *Arthrospira* biomass and then sun-dry the product. Cost estimates indicate that the product can be sold for less than US$ 6 kg^{-1}. This is a very important step towards the production of *Arthrospira* biomass, not only as a health food product, but for a much larger market such as for feed additives. Further reduction of production cost depends upon several factors: (1) increase in

Fig. 25.17 (**a**) An overview of the soda Lake Kossorom (Chad); (**b**) a bloom of *Arthrospira fusiformis* (Lake Kossorom); (**c**) accumulation of *A. fusiformis* biomass at the edge of the lake; (**d**) harvesting *A. fusiformis* blooms from the surface of Lake Kossorom; (**e**) young women collecting pieces of sun-dried *Arthrospira* biomass (*dihé*) after drying them on sand (Photos M.R. Tredici)

productivity of the organism; (2) control of contamination and hence the reduction on the culture renewable time; (3) increasing of harvesting efficiency; (4) reduction of the overall operational efficiency of the farm (Shimamatsu 2004).

25.7 Market and Application

The biomass produced is mainly sold to the health food additives market in the form of powder or pills. It has also been selected by the NASA (National Aeronautics and Space Administration) and by the European Space Agency as one of the primary foods for long-term space missions. In many cases the term "Spirulina" has become a practical synonym for cultivated microalgae. Attempts have been made by Proteous (a marketing company mainly associated with Earthrise Farms in the USA) to incorporate *Arthrospira* biomass into a variety of food products such as granola bars and various kinds of pasta. In Mexico and China, subsidized by the government, *Arthrospira* powder is added to child food such as biscuits and chocolate (Fox 1985). Another available product is a protein extracted from *Arthrospira* biomass containing mainly the blue pigment phycocyanin and marketed under the "Lima Blue" brand name (Dainippon Ink 1980, 1981). The product is mainly used as a colourant for the food market to provide an edible dye for ice cream and as a natural dye in the cosmetics industry. The main problem is that the pigment is light sensitive and special care has to be taken in handling the dye to protect it from bleaching. Full accounts of the potential application of *Arthrospira* as a nutritional and therapeutic supplement in health management are given by Belay (2002) and Gershwin and Belay (2008).

Beside its commercial production, *Arthrospira* still has an important role as food in the economy of some African

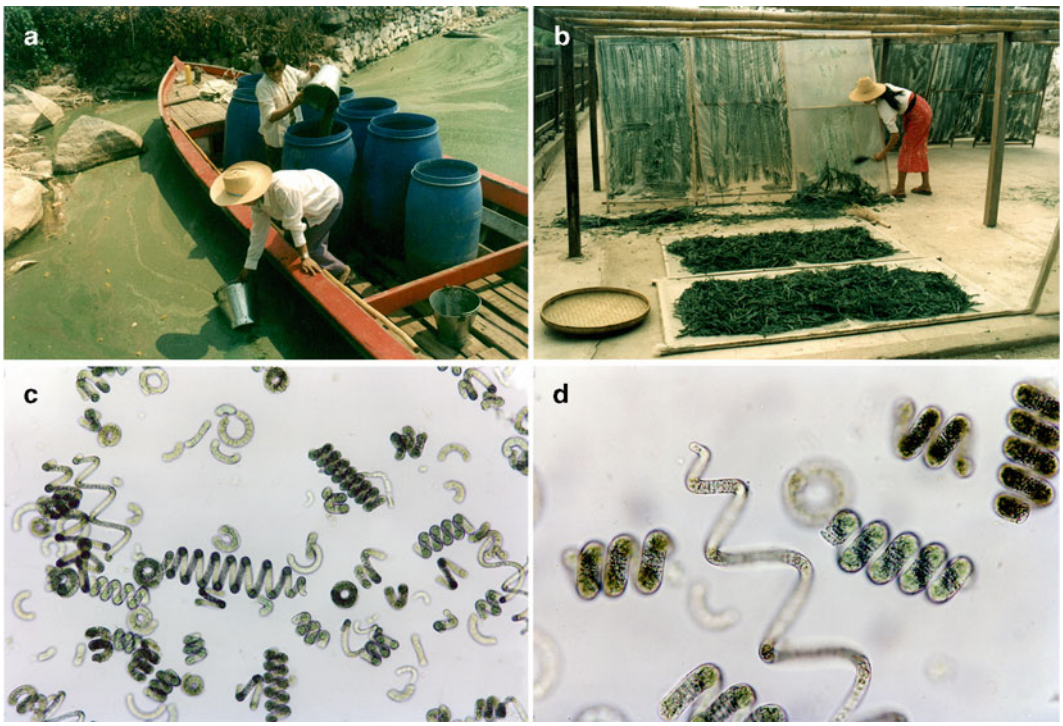

Fig. 25.18 (**a**) Harvesting *Arthrospira fusiformis* from the surface of Twin Taung Lake, Myanmar. (**b**) Noodle-like filaments of *Arthrospira* paste dried in the sun on transparent plastic sheets. (**c**, **d**) Sun-dried trichomes of *Arthrospira fusiformis* (Photos **a**, **b**: A. Vonshak; **c**, **d**: C. Sili)

countries. In 2000 a delegation funded by the African Development Bank visited a number of African countries in an attempt to study to what extent the local tribes use *Arthrospira* biomass as a part of their local food in order to supplement their protein requirements (Sodelac 2000). The internal report issued indicated that the harvesting of naturally-occurring blooms of *Arthrospira* from lakes around the Rift Valley is a very common tradition. A well-defined social and practical structure has been reported. Only Kanembu women carry out the harvesting, while men are banned from entering the water, since it is a deep-rooted belief that they would make the lake barren. *Arthrospira* biomass is harvested from Lake Kossorom throughout the year, with minimum yield in December and January, and a maximum from June to September during the rainy season. The sun-dried product is used by the people who harvest it, as well as being sold on many local markets. It has been estimated that up to 4,000 t dried *Arthrospira* biomass are harvested every year by local tribes in different countries from 14 wadis flooded by Lake Chad during the rainy season. According to Sodelac (2000) in 2000, over 90% of the production was sold by female producers. The price of traditional *dihé* at production sites in 2009 ranged between US$ 0.678 and 1.1 kg^{-1}.

A survey carried out among the Kanembu who harvest *Arthrospira* from Lake Kossorom in the Prefecture of Lac (Chad) has added considerable understanding of local economics (Abdulqader et al. 2000). The trading value of the *dihé* annually harvested from Lake Kossorom (about 40 t) amounted to more than US $ 100,000 which represents an important income for the economy of the area (Fig. 25.17). Natural production of *Arthrospira* has been also documented in Myanmar. Four volcanic lakes with natural *Arthrospira* bloom were studied beginning in 1984. Production began at Twin Taung Lake in 1988, and in 1999 increased to 100 t $year^{-1}$. About 60% is harvested from boats on the surface of the lake, while the other 40% is cultivated in outdoor ponds alongside the lake. During the bloom season in summer, when *Arthrospira* forms thick mats on the lake, people in boats collect a dense concentration of *Arthrospira* in buckets (Fig. 25.18a). This biomass is harvested on inclined filters, washed with fresh water, dewatered and pressed again. The paste is then extruded into noodle-like filaments which are dried in the sun on transparent plastic sheets (Fig. 25.18b). Dried chips are taken to a pharmaceutical factory in Yangoon, pasteurized, and pressed into tablets (Habib et al. 2008). A more detailed study on the consumption patterns in these various countries, as well as on the strains used, could A more detailed study on the consumption patterns, as well as on the strains used, could provide valuable information for the further development of the *Arthrospira* production, processing and consumption industry.

The accumulated knowledge of the ecology, physiology and biochemistry of *Arthrospira* has made its commercial production feasible. At present the photosynthetic efficiency

of *Arthrospira* is still well below (2–3%) of the theoretical maximum, which can be set at about 10% total solar light. Light saturation it has indicated to be one of the most important factors which limit the full exploitation of the photosynthetic capacity. More work is required to provide strain selection and better understanding of the factors governing growth, especially light utilization in dense cultures, to support this growing agro-industry.

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References

Abdulqader G, Barsanti L, Tredici M (2000) Harvest of *Arthrospira platensis* form Lake Kossorom (Chad) and its household usage among the Kanembu. J Appl Phycol 12:493–498

Abeliovich A, Azov Y (1976) Toxicity of ammonia to algae in sewage oxidation ponds. Appl Environ Microbiol 31(6):801–806

Aikawa S, Izumi Y, Matsuda F, Hasunuma T, Chang JS, Kondo A (2012) Synergystic enhancement of glycogen production in *Arthrospira platensis* by optimization of light intensity and nitrate supply. Bioresour Technol 108: 211–215

Alcocer J, Williams WD (1996) Historical and recent changes in Lake Texoco, a saline lake in Mexico. Int J Salt Lake Res 5:45–61

Aldea R, Guțu A, Nicoară A, Dragoş N (2002) Genetic variation of *Arthrospira* (Spirulina) strain assessed by PCR techniques based on genomic repetitive sequences. Contributii Botanice 37:127–136

An J, Carmichael WW (1996) Technical booklet for the microalgae biomass industry: detection of microcystins and nodularins using an enzyme linked immunosorbant assay (ELISA) and a protein phosphatase inhibition assay (PPIA). Department of Biological Sciences, Wayne State University, Dayton, OH

Asada K (1994) Mechanisms for scavenging reactive molecules generated in chloroplasts under light stress. In: Baker NR, Bowyer JR (eds) Photoinhibition of photosynthesis from molecular mechanisms to the field. Bios Scientific Publishers, Oxford, pp 129–142

Averdano MC, Maeso ES, Nieva M, Fernandez Valiente E (1989) Effect of sodium deficiency on the ultrastructure of the cyanobacterium Anabaena PCC 7119. J Plant Physiol 135:409–415

Balloni W, Tomaselli L, Giovannetti L, Margheri MC (1980) Biologia fondamentale del genere *Spirulina*. In: Materassi R (ed) Prospettive della Coltura Massiva di *Spirulina* in Italia. CNR Rome, pp 49–85

Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf JS, Codd GA, Pflugmacher S (2004) Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya – Lakes Bogoria, Nakuru and Elmenteita. J Plankton Res 26:925–953

Ballot A, Krienitz L, Kotut K, Wiegand C, Pflugmacher S (2005) Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. Harmful Algae 4:139–150

Ballot A, Kotut K, Novelo E, Krienitz L (2009) Changes of phytoplankton communities in Lakes Naivasha and Oloidien, examples of degradation and salinization of lakes in the Kenyan Rift Valley. Hydrobiologia 632:359–363

Bao Y, Liu M, Wu X, Cong W, Ning Z (2012) *In situ* carbon supplementation in large-scale cultivations of *Spirulina platensis* in open raceway pond. Biotechnol Bioprocess Eng 17:93–99

Baurain D, Renquin L, Grubisic S, Scheldeman P, Belay A, Wilmotte A (2002) Remarkable conservation of internally transcribed spacer sequences of *Arthrospira* ("*Spirulina*") (Cyanophyceae, Cyanobacteria). Strains from four continents and of recent and 30-years old samples from Africa. J Phycol 38:384–393

Belay A (2002) The potential application of *Spirulina (Arthrospira)* as a nutritional and therapeutic supplement in health management. J Am Nutra Assoc (JANA) 5(2):27–48

Belay A (2008) Spirulina (*Arthrospira*): production and quality assurance. In: Gershwin ME, Belay A (eds) Spirulina in human nutrition and health. CRC Press/Taylor & Francis Group, London, pp 1–25, 312 pp

Belkin S, Boussiba S (1991) Resistance of *Spirulina platensis* to ammonia at high pH values. Plant Cell Physiol 32:953–958

Bergman AN, Laurent P, Otiang'a-Oqwiti G, Bergman HL, Walsh PJ, Wilson P, Wood CM (2003) Physiological adaptation of the gut in the Lake Magadi tilapia, Alcolapia grahami, an alkaline-ad saline adapted teleost fish. Comp Biochem Physiol A 136:701–715

Bernard Ch (1909) Sur quelques algues unicellulaires d'eau douce récoltés dans le Domaine Malais. Dep Agric Indes Néerlandaises, Buitzorg, 94 pp

Blumwald E, Tel-Or E (1982) Osmoregulation and cell composition in salt adaptation of *Nostoc muscorum*. Arch Microbiol 132:168–172

Blumwald E, Mehlhorn RJ, Packer L (1984) Salt adaptation mechanisms in the cyanobacterium *Synechococcus* 6311. In: Sybesma S (ed) Advances in photosynthesis research. Martinus Nijhoff/Dr W Junk Publs, The Hague, pp 627–630

Bocci F, Ferrari F, Materassi R, Mannelli D (1980) Prime ricerche sulla crescita di *Spirulina platensis* e *Spirulina maxima* in coltura fotolimitata. In: Atti XIX Congr Naz Soc Ital Microbiol, Catania (Italy) July 5–8

Booker MJ, Walsby AE (1979) The relative form resistance of straight and helical blue-green algal filaments. Br Phycol J 14:141–150

Borowitzka LJ (1986) Osmoregulation in blue-green algae. In: Round FE, Chapman DJ (eds) Progress in phycological research, vol 4. Biopress Ltd, Bristol, pp 243–256

Bourrelly P (1970) Les Algues d'Eau Douce. III Les Algues bleues et rouges, les Eugléniens, Peridiniens et Cryptomonadines. Editions N Boubée & Cie, Paris, 512 pp

Boussiba S (1989) Ammonium uptake in the alkalophilic cyanobacterium *Spirulina platensis*. Plant Cell Physiol 32:303–314

Boussiba S, Gibson J (1991) Ammonia traslocation in cyanobacteria. FEMS Microbiol Lett 88:1–14

Boussiba S, Affalo C (2005) An insight into the future of microalgal biotechnology. Innov Food Technol 28:37–39

Brandily MY (1959) Depuis des lustres une tribu primitive du Tchad exploite la nourriture de l'an 2000. Sci Avenir 152:516–519

Busson F (1971) *Spirulina platensis* (Gom.) Geitler et *Spirulina geitleri* J De Toni, Cyanophycées Alimentaires. Service de Santé, Marseille

Campbell D, Hurry V, Clarke A, Gustafsson P, Oquist G (1998) Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. Microbiol Mol Biol Rev 6:667–683

Carr NG, Whitton BA (eds) (1973) The biology of blue-green algae. Blackwell Scientific Publications, Oxford, 676 pp

Carr NG, Whitton BA (eds) (1982) The biology of cyanobacteria. Blackwell Scientific Publications, Oxford, 688 pp

Castenholz RW (1989) Subsection III, order oscillatoriales. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams and Wilkins Co, Baltimore, pp 1771–1780

Castenholz RW (2001) Form-genus I *Arthrospira* Stizenberger 1852. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 542–543

Ciferri O (1983) *Spirulina*, the edible microorganism. Microbiol Rev 47:551–578

Clément G (1968) A new type of food algae. In: Mateles RJ, Tannenbaum SR (eds) Single-cell protein. Massachusetts Institute of Technology Press, Cambridge, MA

Coe MJ (1966) The biology of *Tilapia grahami* Boulenger in Lake Magadi, Kenya. Acta Trop 23:146–177

Colwell RR (1970) Polyphasic taxonomy of the genus *Vibrio*: numerical taxonomy of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and related *Vibrio* species. J Bacteriol 104:410–433

Compère P (1974) Cyanophycées de la région du lac Tchad, taxons, combinaisons et noms nouveaux. Bull Jard Bot Nat Belg 44: 17–21

Dadheech PK, Ballot A, Casper P, Kotut K, Novelo E, Lemma B, Pröschold T, Krienitz L (2010) Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Oscillatoriales, Cyanobacteria) of African, Asian and America origin deduced by 16S-23S ITS and phycoyanin operon sequences. Phycologia 49(4):361–372

Dainippon Ink & Chemicals Inc. (1980) Production of highly purified alcoholophilic phycocyanin. Japanese Patent 80 77, 890

Dainippon Ink & Chemicals Inc. (1981) Cosmetics containing water soluble phycocyanin. Japanese Patent 7 9–138755

Dangeard P (1940) Sur une algue bleue alimentaire pour l'homme: *Arthrospira platensis* (Nordst.) Gomont. Act Soc Linn Bordx 91:39–41

De Toni GB (1935) Noterelle di nomenclatura algologica 3:1–2

Desikachary TV (1959) Cyanophyta. Indian Council of Agricultural Research, New Delhi, pp 187–198, 686 pp

Desikachary TV, Jeeji Bai N (1992) Taxonomic studies in *Spirulina*. In: Seshradi CV, Jeeji Bai N (eds) *Spirulina* ETTA national symposium. MCRC, Madras, pp 12–21

Desikachary TV, Jeeji Bai N (1996) Taxonomic studies in *Spirulina* II. The identification of *Arthrospira* ("*Spirulina*") strains and natural samples of different geographical origins. Arch Hydrobiol Suppl 116 Algol Stud 83:163–168

Ehrenfeld J, Cousin JL (1984) Ionic regulation of the unicellular green alga *Dunaliella tertiolecta*: response to hypertonic shock. J Membr Biol 77:45–55

El-Bestawy E, Bellinger EG, Sigee DC (1996) Elemental composition of phytoplankton in a subtropical lake: X-ray microanalytical studies on the dominant algae *Spirulina platensis* (Cyanophyta) and *Cyclotella meneghiniana* (Bacillariophyceae). Eur J Phycol 31:157–166

Espie GS, Miller AG, Canvin DT (1988) Characterization of the Na^+-Requirement in cyanobacterial photosynthesis. Plant Physiol 88:757–763

Fathi AA, Abdelzaher A, Flower RJ, Ramdani M, Kraïem MM (2001) Phytoplankton communities of North African wetland lakes: the CASSARINA Project. Aquatic Ecology 35:303–318

Ferreira LS, Rodrigues MS, Converti A, Sato S, Carvalho JCM (2012) *Arthrospira (Spirulina) platensis* cultivation in tubular photobioreactor: use of no-cost CO_2 from ethanol fermentation. Appl Energy 92:379–385

Fogg GE, Stewart WDP, Fay P, Walsby AE (1973) The blue-green algae. Academic, London, 459 pp

Fott B, Karim AGA (1973) *Spirulina* plankton community in a lake in Jebel Marra, Sudan. Arch Protistenk 115:408–418

Fox DL (1976) Animal biochromes and structural colours, 2nd edn. University of California Press, Berkeley, p 433

Fox RD (1985) Spirulina, the alga that can end malnutrition. Futurist 19:30–35

Fox RD (1996) *Spirulina* production and potential. Edisud, Aix-en-Provence

Fry IV, Huflejt M, Erber WWA, Peshek GA, Packer L (1986) The role of respiration during adaptation of the freshwater cyanobacterium *Synechococcus* 6311 to salinity. Arch Biochem Biophys 244: 686–690

Fuzinato S, Fodora A, Subakov-Simic G (2010) *Arthrospira fusiformis* (Voronichin) Komárek et Lund (Cyanoprocariota)- A new species for Europe. Algol Stud 134:17–24

Gallegos AJ (1993) The past, present and future of algae in Mexico. Bull Inst Océanograph Monaco 12:133–139

Gao K, Li P, Watanabe T, Helbling EW (2008) Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta). J Phycol 44:777–786

Gardner NL (1917) New Pacific coast marine algae I. Univ Calif Publ Bot 6:377–416, Plates 31–35

Geitler L (1925) Cyanophyceae. In: Pascher Süsswasserflora 12. G Fisher-Verlag, Jena, 450 pp

Geitler L (1932) Cyanophyceae. In: Rabenhorst's Krytogamen-Flora von Deutschland, Österreich und der Schweiz 14. Akademische Verlagsgesellschaft, Leipzig, 1196 pp

Gershwin ME, Belay A (2008) In: Gershwin ME, Belay A (eds) *Spirulina* in human nutrition and health. CRC Press/Taylor & Francis Group, Boca Raton

Gomont M (1892) Monographie des Oscillariées. Ann Sci Nat Bot, Sér 7, 15:263–368; 16:91–264

Gong H, Tang Y, Wang J, Wen X, Zhang L, Lu C (2008) Characterization of photosystem II in salt stressed cyanobacterial Spirulina platensis cells. Biochim Biophys Acta 1777:488–495

Grant WD (2006) Alkaline Environments and Biodiversity. In: Gerday C, Glansdorff N (eds) Extremophiles. In Encyclopedia of Life Support Systems (EOLSS). Eolss Publishers, Oxford. htpp://www.eolss.net

Grant WD, Mwatha WE, Jones BE (1990) Alkaliphiles: ecology, diversity and applications. FEMS Microbiol Rev 75:255–270

Greque de Morais M, Da Cruze Reichert C, Dalcaton F (2008) Isolation and characterization of a new *Arthrospira* strain. Z Naturforsch 63c:144–150

Guglielmi G, Cohen-Bazire G (1982) Structure et distribution des pores et des perforations de l'enveloppe de peptidoglycane chez quelques cyanobactéries. Protistologica 18:151–165

Guterman H, Vonshak A, Ben-Yaakov S (1989) Automatic on-line growth estimation method for outdoor algal biomass production. Biotechnol Bioeng 34:143–152

Habib B, Parvin, Huntington TC, Hasan MR (2008) A review on culture, production and use of Spirulina as food for humans and feeds for domestic animal and fish. FAO Fisheries and Aquaculture Circular No. 1034, ISBN 978-92-5-106-106-0

Hindák F (1985) Morphology of trichomes in *Spirulina fusiformis* Voronichin from Lake Bogoria, Kenya. Arch Hydrobiol Suppl 71 Algol Stud 38(39):209–218

Hongsthong A, Sirijuntarut M, Prommeenate P, Thammathorn S, Bunnag B, Cheevadhanarak S, Tanticharoen M (2007) Revealing differentially expressed proteins in two morphological forms of *Spirulina platensis* by proteomic analysis. Mol Biotechnol 36:123–130

Horikoshi K, Akiba T (1982) Alkalophilic microorganisms. Springer, Berlin

Iltis A (1968) Tolérance de salinité de *Spirulina platensis* (Gom.) Geitler, (*Cyanophyta*) dans les mares natronées du Kanem (Tchad). Cah ORSTOM, sér Hydrobiol 2:119–125

Iltis A (1969a) Phytoplancton des eaux natronées du Kanem (Tchad). I. Les lacs permanents a Spirulines. Cah ORSTOM, sér Hydrobiol 3:29–44

Iltis A (1969b) Phytoplancton des eaux natronées du Kanem (Tchad). II. Les mares temporaires. Cah ORSTOM, sér Hydrobiol 3:3–19

Iltis A (1970) Phytoplancton des eaux natronées du Kanem (Tchad). III. Variations annuelles du plancton d'une mare temporaire. Cah ORSTOM, sér Hydrobiol 4:53–59

Iltis A (1971) Phytoplancton des eaux natronées du Kanem (Tchad). V. Les lacs mésohalins. Cah ORSTOM, sér Hydrobiol 5:73–84

Iltis A (1972) Algues des eaux natroné du Kanem (Tchad). Primo. Premiere saltie. Cah ORSTOM, sér Hydrobiol 4:129–134

Iltis A, Riou-Duwat S (1971) Variations saisonnières du peuplement en rotifères des eaux natronées du Kanem (Tchad). Cah ORSTOM, sér Hydrobiol 5:101–112

Jeeji Bai N (1985) Competitive exclusion or morphological transformation? A case study with *Spirulina fusiformis*. Arch Hydrobiol Suppl 71 Algol Stud 38(39):191–199

Jeeji Bai N (1999) A taxonomic appraisal of the genera *Spirulina* and *Arthrospira*. In: Mahato AK, Vidyavati (eds) Recent trends in algal taxonomy, vol I, Taxonomical issues. APC Publications PVT LTD, New Delhi, pp 253–272

Jeeji Bai N, Seshadri CV (1980) On coiling and uncoiling of trichomes in the genus *Spirulina*. Arch Hydrobiol Suppl 60 Algol Stud 26:32–47

Kebede E, Ahlgren G (1996) Optimum growth conditions and light utilisation efficiency of *Spirulina platensis* (equal *Arthrospira fusiformis*) (Cyanophyta) from Lake Chitu, Ethiopia. Hydrobiologia 332:99–109

Kerfeld CA (2004) Structure and function of the water-soluble carotenoid-binding proteins of cyanobacteria. Photosynth Res 81:215–225

Kirilovsky D (2007) Photoprotection in cyanobacteria: the orange carotenoid protein (OCP)-related non-photochemical-quenching mechanism. Photosynth Res 93:7–16

Komárek J, Anagnostidis K (2005) Cyanoprokaryota – Part 2: oscillatoriales. In: Büdel B, Krienits L, Gärtner G, Schagerl M (eds) Süsswasserflora von Mitteleuropa 19/2. Elsevier/Spektrum, Heidelberg, pp 341–353, 759 pp

Komárek J, Hauer T (2011) CyanoDB.cz – On-line database of cyanobacterial genera. – Word-wide electronic publication, University of South Bohemia & Institute of Botany AS CR. http://www.cyanodb.cz

Komárek J, Lund JWG (1990) What is "*Spirulina platensis*" in fact? Arch Hydrobiol Suppl 85 Algol Stud 58:1–6

Krause GH (1994) The role of oxygen in photoinhibition of photosynthesis. In: Foyer CH, Mullinex PM (eds) Cause of photooxidative stress and amelioration of defences systems in plants. CRC Press, Boca Raton, pp 42–76

Laliberté G, Olguín EJ, de la Noüe J (1997) Mass cultivation and wastewater treatment using *Spirulina*. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): physiology, cell-biology and biotechnology. Taylor & Francis Ltd, London, pp 159–173, 233 pp

Lee YK (1997) Commercial production of microalgae in the Asia-Pacific rim. J Appl Phycol 9:403–4511

Léonard J, Compère P (1967) *Spirulina platensis* (Gom.) Geitler, algue bleue de grande valeur alimentaire par sa richesse en protéines. Bull Jard Bot Nat Belg 37:1–23

Leverenz JW, Falk S, Pilström CM, Samuelson G (1990) The effect of on the photosynthetic light-response curve of green plant cells (*Chlamydomonas reinhardtii*). Planta 182:161–168

Lewin RA (1980) Uncoiled variants of *Spirulina platensis* (Cyanophyceae: Oscillatoriaceae). Arch Hydrobiol Suppl 60 Algol Stud 26:48–52

Li BS, Qiao V, Tian XY (2003) Potential to development *Spirulina platensis* with alkaline lakes in Orodos Plateau. Plant Mag 06:18–20 (cited by Lu et al. 2011)

Lincoln EP, Hall TW, Koop B (1983) Zooplankton control in mass algal cultures. Aquaculture 32:331–337

Long SP, Humphries S, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Annu Rev Plant Physiol Plant Mol Biol 45:633–662

Lu C, Vonshak A (2002) Effects of salinity on photosystem II function in cyanobacterial *Spirulina platensis* cells. Physiol Plant 114:405–413

Lu C, Torzillo G, Vonshak A (1999) Kinetic response of photosystem II photochemistry in the cyanobacterium *Spirulina platensis* to high salinity is characterised by two distinct phases. Aust J Plant Physiol 26:283–292

Lu Y-M, Xiang W-Z, Wen Y-H (2011) *Spirulina* (*Arthrospira*) industry in Inner Mongolia of China: current status and prospects. J Appl Phycol 23:264–269

Ma Z, Gao K (2009) Photoregulation of morphological structure and its physiological relevance in the cyanobacterium *Arthrospira* (*Spirulina*) *platensis*. Planta 230:329–337

Maglione G. (1969) Premières données sur le regime hydrogéochimique des lacs permanents du Kanem (Tchad). Cah ORSTOM, sér Hydrobiol 3:121–141

Manen JF, Falquet J (2002) The cpcB-cpcA locus as a tool for the genetic characterization of the genus *Arthrospira* (Cyanobacteria): evidence for horizontal transfer. Int J Syst Evol Microbiol 52:861–867

Margheri MC, Tomaselli Feroci L, Materassi R (1975) Contributo sperimentale alla sistematica del genere *Spirulina* Turpin. In: Atti XVII Cong Naz Soc Ital Microbiol, Padova, vol II, pp 891–901

Marquez FJ, Sasaki K, Nishio N, Nagai S (1995) Inhibitory effect of oxygen accumulation on growth of *Spirulina platensis*. Biotechnol Lett 17:222–228

Martel A, Yu S, Garcia-Reina G, Lindblad P, Pedersén M (1992) Osmotic-adjustment in the cyanobacterium *Spirulina platensis*: presence of an α-glucosidase. Plant Physiol Biochem 30:573–578

Melack JM (1979) Photosynthesis and growth of *Spirulina platensis* (Cyanophyta). In an equatorial lake (Lake Simbi, Kenya). Limnol Oceanogr 24:753–760

Miller AG, Turpin DH, Canvin DT (1984) Na^+ Requirement for growth, photosynthesis, and pH regulation in the alkalotolerant cyanobacterium *Synechococcus leopoliensis*. J Bacteriol 159:100–106

Molitor V, Erber W, Peschek GA (1986) Increased levels of cytochrome oxidase and sodium proton antiporter in the plasma membrane of *Anacystis nidulans* after growth in sodium enriched media. FEBS Lett 204:251–254

Mühling M, Harris N, Belay A, Whitton BA (2003) Reversal of helix orientation in the cyanobacterium *Artrhospira*. J Phycol 39:360–367

Mühling M, Somerfield PJ, Harris N, Belay A, Whitton BA (2006) Phenotypic analysis of *Arthrospira* (Spirulina) strains (cyanobacteria). Phycologia 45(2):148–157

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007) Photoinhibition of PSII under environmental stress. Biochim Biophys Acta:1767:414–421

Mussagy A, Wilmotte A, Cronberg G (2006) The cyanophyte *Arthrospira fusiformis* from Mozambique, Africa: morphological and molecular characterization. Algol Stud 121:59–73

Noor P, Akhtar N, Munshi JL, Begum S (2008) *Spirulina* culture in Bangladesh XII. Effects of different culture media, different culture vessels and different cultural conditions on coiled and straight filament characteristics of *Spirulina*. Bangladesh J Sci Ind Res 43(3):369–376 (Available at www.banglajol.info)

Oberholster PJ, Botha AM, Ashton PJ (2009) Appearance of new taxa: invertebrates phytoplankton and bacteria in a alkaline, saline, meteorite crater lake, South Africa. Fundam Appl Limnol Archiv Hydrobiol 173. doi:10.1127/1863-9135/2009

Ogawa T, Aiba S (1978) CO_2 assimilation and growth of a blue-green alga, *Spirulina platensis*, in continuous culture. J Appl Chem Biotechnol 28:5151–5157

Ohmori K, Ehira S, Kimura S, Ohmori M (2009) Changes in the amount of cellular trehalose, the activity of maltooligosyl trehalose hydrolase, and the expression of its gene in response to salt stress in the cyanobacterium *Spirulina platensis*. Microbes Environ 24(1):52–56

Okoth OE, Mucai M, Shivoga WA, Miller SN, Rasowo J, Ngugi CC (2009) Spatial and seasonal variations in phytoplankton community structure in alkaline-saline Lake Nakuru, Kenya. Lake & Reservoirs. Res Manag 14:57–69

Padan E, Zilberstein D, Schuldiner S (1981) pH Homeostasis in bacteria. Biochim Biophys Acta 650:151–166

Padisák J, Soróczki-Pintér E, Rezner Z (2003) Sinking properties of some phytoplankton shapes and the relation of form resistance to morphological diversity of plankton – an experimental study. Hydrobiologia 500:243–257

Palinska KA, Krumbein WE (2000) Perforation patterns in the peptidoglycan wall of filamentous cyanobacteria. J Phycol 36:139–145

Peschek GA, Obinger C, Fromwald S, Bergman B (1994) Correlation between immuno-gold labels and activities of cytochrome-c oxidase (aa-type) in membranes and salt stressed cyanobacteria. FEMS Microbiol Lett 124:431–438

Pogoryelov D, Sudhir P-R, Kovács L, Gombos Z, Brown I, Garab G (2003) Sodium dependency of the photosynthetic electron transport in the alkaliphilic cyanobacterium *Arthrospira platensis*. J Bioenerg Biomembr 35(5):427–437

Qiao C, Li BS, Zeng ZQ (2001) Alkaline lakes and *Spirulina* (*Arthrospira*) resources in sandy land of Erdos. J Arid Land Res Environ 15(4):86–91

Ramus J (1981) The capture and transduction of light energy. In: Lobban CS, Wynne MJ (eds) The biology of seaweeds, botanical monographs, vol 17. Blackwell Scientific Publications, Oxford, pp 458–492

Reed RH, Richardson DL, Stewart WDP (1985) Na^+ uptake and extrusion in the cyanobacterium *Synechocystis* PCC 6714 in response to hypersaline treatment. Evidence for transient changes in plasmalemma Na^+ permeability. Biochim Biophys Acta 814:347–353

Rich F (1931) Notes on *Arthrospira platensis*. Rev Algol 6:75–82

Rich F (1932) Report on the Percy Sladen Expedition to some Rift Valley Lakes in Kenya in 1929. IV. Phytoplankton from the Rift Valley Lakes in Kenya. Ann Mag Nat Hist, ser.10, 10:233–262

Richmond A (2004a) In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd, Oxford, 566 pp

Richmond A (2004b) Biological principles of mass cultivation. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd, Oxford, pp 125–177

Rippka R, Herdman M (1992) Catalogue of strains. Pasteur culture collection of cyanobacterial strains in axenic culture. Institut Pasteur, Paris, 103 pp

Rodrigues MS, Ferreira LS, Converti A, Sato S, Monteiro de Carvalho JC (2011) Influence of ammonium sulphate feeding time on the fed-batch *Arthrospira (Spirulina) platensis* cultivation and biomass composition with and without pH control. Bioresour Technol 102:6587–6592

Scheldeman P, Baurain D, Bouhy R, Scott M, Mühling M, Whitton BA, Belay A, Wilmotte A (1999) *Arthrospira* ('*Spirulina*') strains from four continents are resolved into two only two clusters, based on ARDRA (Amplified Ribosomal DNA Restriction Analysis) of the ITS (Internal Transcribed Spacer). FEMS Microbiol Lett 172:213–222

Schlesinger P, Belkin S, Boussiba S (1996) Sodium deprivation under alkaline conditions causes rapid death of the filamentous cyanobacterium *Spirulina platensis*. J Phycol 32:608–613

Shimamatsu H (2004) Mass production of *Spirulina*, an edible microalga. Hydrobiologia 512:39–44

Sili C, Abdulqader G, Tredici MR (1999) Photosynthetic biocenosis of two soda lakes of the north-east fringe of Lake Chad. In Abstracts of the 8th international conference of applied algology "Algae and human affairs in the 21st century", Montecatini Terme, p 318

Sodelac (2000) Etude de préfaisabilité du developpement de la production de spirulines. Répubblique du Tchad. Ministére de l'Agriculture. Tractebel Consult (Belgique). Financement: Fond African de Dèveloppement. Contract N 003/SODELA C/99

Spiller S, Denbeaux G, Jones G, Pearson AL (2000) Fine structure of cyanobacteria, *Spirulina platensis* and *Spirulina subsalsa*, as viewed by x-ray microscope, XM-1, beamline 6.1.2. In Compendium 2000. The Advanced Light Source (ALS), Lawrence Berkeley National Laboratories, General ALS Publications

Stizenberger E (1852) *Spirulina* und *Arthrospira* (nov. gen.). Hedwigia 1:32–41

Talling JF, Talling IB (1965) The chemical composition of African lake waters. Int Rev Hydrobiol 50:1–32

Thein M (1993) Production of *Spirulina* in Myanmar. Bull Inst Océanograph Monaco 12:175–178

Tomaselli L (1997) Systematic and ecology. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): physiology, cell-biology and biotechnology. Taylor & Francis Ltd, London, pp 1–19, 233 pp

Tomaselli L, Giovannetti L, Margheri MC (1981) On the mechanism of trichome breakage in *Spirulina platensis* and *S. maxima*. Ann Microbiol 31:27–31

Tomaselli L, Torzillo G, Giovannetti L, Pushparaj B, Bocci F, Tredici M, Papuzzo T, Balloni W, Materassi R (1987) Recent research on *Spirulina* in Italy. Hydrobiologia 151(152):79–82

Tomaselli L, Giovannetti L, Sacchi A, Bocci F (1988) Effects of temperature on growth and biochemical composition in *Spirulina platensis* strain M2. In: Staedler T, Mollion J, Verdus MC, Karamanos Y, Morvan H, Christiaen D (eds) Algal biotechnology. Elsevier Applied Sciences, London/New York, pp 305–314

Tomaselli L, Giovannetti L, Torzillo G (1993) Physiology of stress response in *Spirulina* spp. Bull Inst Océanograph, Monaco 12:65–75

Tomaselli L, Palandri MR, Tredici MR (1996) On the correct use of the *Spirulina* designation. Arch Hydrobiol Suppl 116 Algol Stud 83:539–548

Thomasson K (1960) Ett fall av tropisk vattenblomming. [A case of tropical waterbloom] Bot Notiser 113(2):214–216

Torzillo G, Vonshak A (1994) Effect of light and temperature on the photosynthetic activity of the cyanobacterium *Spirulina platensis*. Biomass Bioenergy 6:399–404

Torzillo G, Vonshak A (2003) Biotechnology of algal mass cultivation. In: Fingerman M, Nagabhushanam R (eds) Recent advances in marine biotechnology, vol 9, Biomaterials and bioprocessing. Science Publishers, Inc, Plymouth, pp 45–77, 385 pp

Torzillo G, Giovannetti L, Bocci F, Materassi R (1984) Effect of oxygen concentration on the protein content of Spirulina biomass. Biotechnol Bioeng 26:1134–1135

Torzillo G, Pushparaj B, Bocci F, Balloni W, Materassi R, Florenzano G (1986) Production of *Spirulina* biomass in closed photobioreactors. Biomass 11:61–74

Torzillo G, Sacchi A, Materassi R, Richmond A (1991) Effect of temperature on yield and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors. J Appl Phycol 3:103–108

Torzillo G, Bernardini P, Masojidek J (1998) On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina platensis* (Cyanobacteria). J Phycol 34:504–510

Torzillo G, Komenda J, Kopecky J, Faraloni C, Masojidek J (2003a) Photoinhibitory stress induced by high oxygen and low temperature in outdoor cultures of *Arthrospira platensis* grown in closed photobioreactors. In: Abstracts of third European phycological congress, Belfast, Ireland, July 21–26

Torzillo G, Pushparaj B, Masojidek J, Vonshak A (2003b) Biological constraints in algal biotechnology. Biotechnol Bioprocess Eng 8:338–348

Tredici MR, Papuzzo T, Tomaselli L (1986) Outdoor mass culture of *Spirulina maxima* in sea-water. Appl Microbiol Biotechnol 24:42–50

Tsen CT, Chang TP (1990) Ultrastrukturen von vier "Spirulina"-Arten (Cyanophyceae). Arch Hydrobiol Suppl 87 Algol Stud 60:33–41

Tuite CH (1981) Standing crop densities and distribution of *Spirulina* and benthic diatoms in East African alkaline saline lakes. Freshw Biol 11:345–360

Turpin PJF (1829) *Spirulina oscillarioide*. In: Dictionnaire des Sciences Naturelles, De Lévrault, Paris vol. 50, pp 309–310

Van Eykelenburg C (1977) On the morphology and ultrastructure of the cell wall of *Spirulina platensis*. Antonie Van Leeuwenhoek J Microbiol 43:89–94

Van Eykelenburg C (1979) The ultrastructure of *Spirulina platensis* in relation to temperature and light intensity. Antonie Van Leeuwenhoek J Microbiol 45:369–375

Van Eykelenburg C, Fuchs A (1980) Rapid reversible macromorphological changes in *Spirulina platensis*. Naturwissenschaften 67:200–204

Van Liere L, Mur L (1979) Growth kinetics of *Oscillatoria agardhii* Gomont in continuous culture limited in its growth by the light energy supply. J Gen Microbiol 115:153–159

Vareschi E (1982) The ecology of lake Nakuru (Kenya). III: abiotic factors and primary production. Oecologia 55:81–101

Verma K, Mohanty P (2000) Alterations in the structure of phycobilisomes of the cyanobacterium Spirulina platensis in response to enhanced Na^+ level. World J Microbiol Biotechnol 16:795–798

Viti C, Ventura S, Lotti F, Capolino E, Tomaselli L, Giovannetti L (1997) Genotypic diversity and typing of cyanobacterial strains of the genus *Arthrospira* by very sensitive total DNA restriction profile analysis. Res Microbiol 148:605–611

Vonshak A (1997a) *Spirulina platensis (Arthrospira)*: physiology, cell-biology and biotechnology. Taylor & Francis Ltd, London, 233 pp

Vonshak A (1997b) *Spirulina*: growth, physiology and biochemistry. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): physiology, cell-biology and biotechnology. Taylor & Francis Ltd, London, pp 43–65

Vonshak A, Guy R (1992) Photoadaptation, photoinhibition and productivity in outdoor grown *Spirulina platensis* strains. Plant Cell Environ 15:613–616

Vonshak A, Novoplansky N (2008) Acclimation to low temperature of two *Arthrospira platensis* (cyanobacteria) strains involves down-regulation of PSII and improved resistance to photoinhibition. J Phycol 44:1071–1079

Vonshak A, Richmond A (1988) Mass production of the blue-green alga *Spirulina*: an overview. Biomass 15:233–247

Vonshak A, Richmond A (1981) Photosynthetic and respiratory activity in *Anacystis nidulans* adapted to osmotic stress. Plant Physiol 68:504–509

Vonshak A, Tomaselli L (2000) *Arthrospira* (*Spirulina*) Systematics and ecophysiology. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 505–522, 669 pp

Vonshak A, Abeliovich A, Boussiba S, Arad S, Richmond A (1982) Production of *Spirulina* biomass: effects of environmental factors and population density. Biomass 2:175–185

Vonshak A, Boussiba S, Abeliovich A, Richmond A (1983) Production of *Spirulina* biomass: maintenance of monoalgal culture. Biotechnol Bioeng 25:341–351

Vonshak A, Guy R, Guy M (1988a) The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. Arch Microbiol 150:417–420

Vonshak A, Guy R, Poplawsky R, Ohad I (1988b) Photoinhibition and its recovery in two different strains of *Spirulina*. Plant Cell Physiol 29:721–726

Vonshak A, Chanawongse L, Bunnang B, Tanticharoen M (1996a) Light acclimation and photoinhibition in three *Spirulina platensis* (cyanobacteria) isolates. J Appl Phycol 8:35–40

Vonshak A, Torzillo G, Accolla P, Tomaselli L (1996b) Light and oxygen stress in *Spirulina platensis* (cyanobacteria) grown outdoors in tubular reactors. Physiol Plant 97:175–179

Voronichin NN (1934) Biologie des bassins mineral des steppes Koulondine. Expedition de l'Academie des Sciences d'URSS 1931–1933 I. Leningrad. Trav Soviet pour serv l'étude ressour natur. Sér Siberie 8:182–183

Wang ZP, Zhao Y (2005) Morphological reversion of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta): from linear to helical. J Phycol 41:622–628

Welsh H (1965) A contribution to our knowledge of the blue-green algae of South West Africa and Bechuanaland. Nova Hedwigia 9 (1–4):131–162

Wilson A, Ajilani G, Verbavatz J-M, Vass I, Kerfeld C, Kirilovsky D (2006) A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria. Plant Cell 18:992–1007

Wittrock VB, Nordstedt CFO (1884) *Algae aquae dulcia exiccata*. Fasc 14, 679:59

Wu H, Gao K, Villafañe VE, Watanabe T, Helbling EW (2005) Effects of solar UV radiation on morphology and photosynthesis of filamentous cyanobacterium *Arthrospira platensis*. Appl Environ Microbiol 71(9):5004–5013

Zarrouk C (1966) Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setchell et Gardner) Geitler. Ph D thesis, University of Paris, France

Zeng MT, Vonshak A (1998) Adaptation of *Spirulina platensis* to salinity-stress. Comp Biochem Physiol A 120:113–118

Zhao J, Briand JJ (1988) Sequential events in the photoinhibition of *Synechocystis* under sodium stress. Plant Physiol 91:91–100

Zheng XY, Zhang MG, Dong YH, Gao ZH, Xu C, Han ZM, Zhang BZ, Sun DP, Wang KJ (1992) Salt lakes in Inner Mongolia. Science press, Beijing, pp 41–266 (in Chinese)

The Biotechnology of Cyanobacteria

26

Claudia B. Grewe and Otto Pulz

Contents

Summary

This chapter gives an overview of the range of cyanobacterial materials being harvested from nature and grown in culture, increasingly on a large scale. *Arthrospira*, which is usually marketed as Spirulina, is the most important, but studies are also underway on developing methods to grow *Nostoc* commercially; at present colonies of several species are harvested for local use in a number of countries in Asia, Africa and South America. Although *Aphanizomenon flos-aquae* has been harvested and sold, the costs of the quality control needed to avoid long-term risks of material including toxins makes its large-scale cultivation in photobioreactors preferable. The various approaches to mass culture are considered and the ways in which cyanobacteria are now being used are described. These include food, phycobiliproteins for pigment and antioxidant, animal feed, cosmetics, biofertilizers and treatment of wastewater and exhaust gas. Promising products for the near future include some of the huge range of bioactive molecules produced by cyanobacteria and most important of all, biofuel.

26.1 Introduction

Cyanobacteria are among the oldest and most successful life forms on earth (Chap. 2; Sharma et al. 2010) and their importance in the production of oxygen and fixation of CO_2 has often been stressed (DeRuyter and Fromme 2008). At the same time they are one of the most important primary producers and part of the beginning of the food chain in almost all aquatic habitats; cyanobacterial growth early in the earth's history made a substantial contribution to present-day supplies of crude oil (Chap. 16). However, despite their long evolutionary history, the involvement of cyanobacteria represents one of the newest trends in biotechnology, since much of the focus during the past century has been on bacteria, yeasts and fungi.

The high demand for food, feed and pharmaceuticals has led the development of heterotrophic production processes to

C.B. Grewe (✉) • O. Pulz
Research and Development, Salata GmbH, An der Salzbruecke, 98617 Ritschenhausen, Germany
e-mail: CGrewe@Salata-Salzbruecke.de

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a high level. Extensive screening programmes have been carried out with heterotrophs in the search for bioactive metabolites, so that the re-discovery rate of compounds is already above 90% (Olaizola 2003). At present, energy-consuming processes using heterotrophic organisms are exploited to a much higher degree than autotrophic production processes. Interest is therefore shifting towards other organisms, which are able to produce valuable products in a more sustainable way. Cyanobacteria present a rich resource of biotechnologically important organisms; they can be used both to produce specific molecules and for industrial processes. The biodiversity of cyanobacteria is enormous and represents an almost untapped resource.

The biotechnology of cyanobacteria has gained considerable importance in the last decades, with applications ranging from simple biomass production for food and feed to valuable products. The market size for most of these products continues to increase and the biotechnological use of cyanobacteria will extend into new areas. Considering the vast biodiversity of cyanobacteria and developments in genetic engineering, they represent one of the most promising sources for new products and applications.

The chapter reports on the cyanobacteria which have become relevant in terms of economic applications, their markets as well as the biotechnology behind their production. Only brief mention is made of studies prior to 2000.

26.2 Economically Relevant Cyanobacteria

26.2.1 Overview

Three cyanobacterial genera represent most of the commercially relevant products at present: *Arthrospira*, *Nostoc* and *Aphanizomenon*. These are being produced and/or collected for different purposes, mostly as health food and dietary supplement. All these applications are related to their valuable components and their gross biochemical composition is summarized in Table 26.1. For *Arthrospira* and *Aphanizomenon* a protein content of over 50% dry weight, with a high proportion of essential amino acids is characteristic, though values are lower for *Nostoc*, because of the large amount of extracellular polysaccharide. The lipid content of cyanobacteria is much lower, typically 5–8% (Griffiths and Harrison 2009).

In the case of *Arthrospira*, the average values are above 60% for proteins and 6–8% for lipids, with free fatty acids forming about 50% of these lipids (Gershwin and Belay 2007). β-carotene constitutes 0.14–0.23% of its dry weight, a content 20 times that of carrots, equaling 375,000 IU of vitamin A. Its vitamin B_{12} content is higher than in beef liver, on average 2.5 μg g^{-1} dry weight. The calcium content exceeds the proportion in milk by factor 2.5 (3 mg g^{-1}).

Table 26.1 Gross biochemical composition of commercial relevant cyanobacteria (Schreckenbach et al. 2001; Danxiang et al. 2004; Capelli and Cysewski 2010)

	Arthrospira spp. (%)	*Nostoc* spp. (%)	*Aphanizomenon flos-aquae* (%)
Protein	58–73	10–23	60–75
Lipids (fat)	6–8	5–6	2–8
Carbohydrate	15–25	56–57	20–30

Iron, which is the most common mineral deficiency worldwide, is present in contents up to 2.17 mg g^{-1} exceeding the iron content in spinach by more than 6 times based on dry weight (Capelli and Cysewski 2010). However, any realistic comparison has to acknowledge that people do not consume cyanobacterial biomass in the range of 100 g day^{-1}. The ingredients more relevant to health are of much more interest, such as polysaccharides and antioxidants (e.g. carotenoids). *Aphanizomenon flos-aquae* has a broadly similar composition to *Arthrospira*, being rich in proteins, carotenoids, phycobiliproteins, vitamins and minerals, while *Nostoc flagelliforme* has a higher carbohydrate content but lower protein content (Danxiang et al. 2004), although fewer data are available for the latter. The concentrated nutritional profile makes cyanobacteria in general and *Arthrospira* in particular a valuable nutrient source; *Arthrospira* seems particularly suited to counteract malnutrition (Henrikson 1989).

The exact chemical composition of the biomass depends on environmental conditions, including the source of nutrients and the mode of nutrition (autotrophic, mixotrophic or heterotrophic). Nevertheless considerable consistency has been found in the biochemical composition of *Arthrospira* during the production season, which is remarkable in view of the fact that production is in open systems (Belay 2007). However, production procedures differ between the *Arthrospira* producers and, in addition to cultivation, drying methods and conditions. Packaging and storage can all have an impact on the gross chemical composition of the product, so that the final products of *Arthrospira* biomass can differ markedly in their characteristics (Grobbelaar 2003). Investigations on the chemical composition of *Chlorella* products have shown similar quality differences depending on the production procedures (Görs et al. 2010).

Their interesting product characteristics have stimulated large-scale production of cyanobacterial biomass in the past decade (Fig. 26.1). The amount of *Arthrospira* produced has consistently increased and probably reached 5,000 t dry weight in 2010. Although China joined the producing countries later than many others, it soon became the largest producer worldwide. Over 3,500 t biomass is being produced annually in China by many different companies, with 20% of this on an area of over 500,000 m^2 in Inner Mongolia (Lu et al. 2010). The annual production of the biomass of the

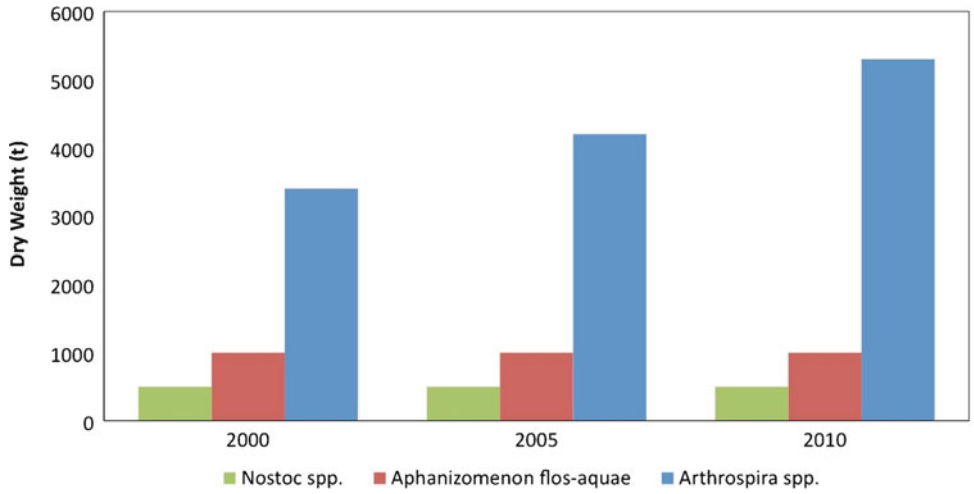

Fig. 26.1 Worldwide production of commercially most relevant cyanobacteria (Available data 2011, Carmichael et al. 2000; Lu et al. 2010)

three cyanobacteria accounts for at least 6,800 t dry weight per year (Fig. 26.1) and thereby 68% of the total worldwide microalgal biomass production of 10,000 t (Rosello Sastre and Posten 2010). The production costs in China of US$ 3–4 per kg (Lu et al. 2010) are lower than those at other production facilities in tropical or subtropical climates. In addition to the large commercial producers, there are efforts to support *Arthrospira* production in Chad: in 2007 the European Union was funding a US$ 1.4 million project run by the UN Food and Agriculture Organization (FAO) in order to support *dihé* production.

Aphanizomenon flos-aquae was harvested in the range of 1,000 t dry weight per year and sold with a market volume of US $100 million (Carmichael et al. 2000). Dried colonies of *Nostoc verrucosum* are consumed in the range of 100 t per year in Asia, especially Myanmar, and are being sold for less than US$ 1 per kg in the local markets in Myanmar (Min Thein, personal communication 2011). In China, prices for *N. flagelliforme* can be as high as US$ 125 per kg (Roney et al. 2009), but other species sell locally for far less in some regions of the country.

For most of the products the whole biomass is used. Increasingly, however, particular ingredients like phycobiliproteins and polysaccharides are being extracted and further purified. These are the subject of some of the following sections of this chapter.

26.2.2 *Arthrospira* (Spirulina)

Arthrospira ("Spirulina") is both the most popular microalga as well as the most extensively studied. *A. platensis* has gained worldwide popularity as a food supplement (Gershwin and Belay 2007), being one of the most protein-rich foods known. As with nearly all microalgae cultivated at present, *A. platensis* is an extremophile, which has many advantages for mass culture (Chap. 25). The alkalophilic organism has the largest stake of cyanobacteria biomass produced worldwide and its unique position will be maintained for the next decades. Although it is not the only cyanobacterial biomass on the market today, it represents the only cyanobacterium that is being extensively cultivated in artificial systems for different applications during the past decades.

Arthrospira shows a cylindrical, loosely or tightly coiled trichome in a regular helix (John et al. 2002). *Arthrospira* is, as many cyanobacteria, a cosmopolitan, being found in many different habitats. Nevertheless alkaline as well as salt containing habitats are preferred. Blooms are observed in bicarbonate-rich environments as well as in high salt concentrations or brackish waters. Trichome breakage depends on necridium formation (Tomaselli 1997; Hu 2004). Its gram-negative, soft cell wall is composed out of four layers, with a major layer of peptidoglycan (Chap. 25).

Arthrospira is regarded as a rich source of vitamins, essential amino acids, minerals, essential fatty acids like y-linolenic acid (GLA, a ω6-polyunsaturated fatty acid) and antioxidant pigments like phycobiliproteins and carotenoids. *A. platensis* and *A. maxima* are apparently the only producers of GLA so far reported for cyanobacteria. This fatty acid is a precursor of arachidonic acid, which is required for the synthesis of important metabolic mediators. The proportion of linoleic, γ-linolenic acid and palmitic acid seem to be species specific within *Arthrospira* (Mühling et al. 2005b) and can aid strain identification. The composition of fatty acids is affected by cultivation conditions: lower temperature, higher light intensity and a change to heterotrophic nutrition favour a higher proportion of PUFAs (Mühling et al. 2005b). GLA is bound to over 94% to glycolipids in the lipid fraction of *A. platensis* (Sajilata et al. 2008a). The carotenoids of *A. maxima* consist mainly of zeaxanthin (25%),

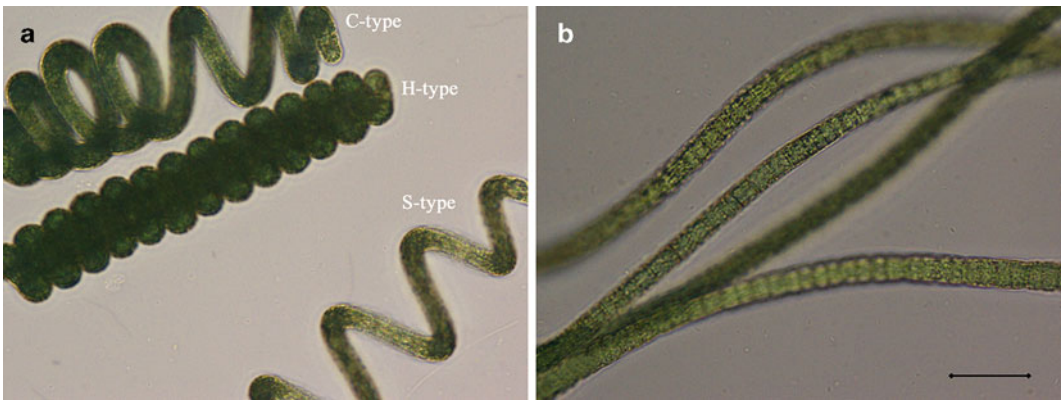

Fig. 26.2 Laboratory culture of *Arthrospira platensis*, (**a**) coiled trichome forms, S-type: loosly coiled, C-type: intermediatly coiled, H-type: tightly coiled; (**b**) straight trichome. Cultures both isolated from Myanmar, Lake of Twin Taung; scale bar = 20 µm

myxoxanthophyll (13–17%), β-carotene (15%), echinenone (11–13%), β-cryptoxanthin (7%) and 3′-hydroxyechinenone (7–11%) (Miki et al. 1986). Studies by Wilson et al. (2008) have shown that high light induces structural changes in a recently identified orange carotenoid protein. This photoactive protein senses blue-green light and triggers photoprotection in cyanobacteria.

Laboratory experiments under autotrophic conditions showed that light saturation in *A. platensis* at 150–200 µmol photon m^{-2} s^{-1}. The original or modifications of the medium of Zarrouk (1966) is widely for culture. The optimal growth temperature is strain dependant, being in the range of 35–38°C, and with a minimum temperature required growth about 15°C in the strains reported by Belay (1997). Its salt amplitude is in the range of a few millimolar up to 0.75 M NaCl. In the latter concentration growth is strain dependant and already inhibited up to 70% (Vonshak and Tomaselli 2000). *Arthrospira* spp. are considered as obligatory alkalophilic organisms, with optimum pH ranges between pH 9.5–12 (Hu 2004). The high pH represents the ecological niche that permits successful cultivation of quite homogeneous cultures of *Arthrospira* in open cultivation systems.

Growth of some strains can occur under mixotrophic and heterotrophic conditions, with a range of substrates reported for various strains: glucose (Chojnacka and Noworyta 2004; Lodi et al. 2005), fructose (Mühling et al. 2005a), acetate (Chen et al. 1996), glycerol (Narayan et al. 2005), propionate (Lodi et al. 2005) and peptone (Vonshak 1997b). Mühling et al. (2005a, b) assayed strains freshly isolated from nature on their heterotrophic growth on different carbon sources, reporting that at least one strain subcultured under autotrophic conditions for a further 2 years had lost the ability to use fructose, emphasizing the influence of strain origin. In addition, mixotrophic tests with ten of the strains from heterotrophic strains showed differences in their response: all made use of glucose and maltose, but none used fructose, even those able to do so under heterotrophic conditions. Mixotrophic tests with sucrose showed rapid lysis of many trichomes, but subsequent recovery of short lengths, followed by continued growth of healthy cultures. Marquez et al. (1993) concluded from studies on *Arthrospira platensis* that in mixotrophic cultivation autotrophic and heterotrophic growth functioned independently during mixotrophic growth.

Use of organic substrates for mixotrophic growth can lead to considerable increases in growth rate compared with autotrophic growth (Mühling et al. 2005a). Mixotrophic cultivation with glucose enhanced growth of *Arthrospira platensis* by a factor of 5.1 (Chen and Zhang 1997), while Lodi et al. reported a 33% higher volumetric cell productivity for *Arthrospira platensis* (Lodi et al. 2005). Not only is the growth rate faster, but the final biomass concentration is higher, enhancing the efficiency of harvesting. In spite of these successes, an upscaling step towards mixotrophic production in commercial systems does not at present seem feasible. This is mainly due to higher costs and microbiological problems; including the likelihood of heterotrophic bacteria and fungi outcompeting *Arthrospira* in the utilization of the organic carbon source.

In general cultivation parameters are influencing the metabolism and the morphology of the algal cells, in the case of *Arthrospira* the change of helix orientation or even the straightening of the trichomes have been observed (Vonshak and Tomaselli 2002; Mühling et al. 2003), see Chap. 25. Nevertheless, this phenomenon occurs both in nature as well as in culture (Fig. 26.2). It has not been satisfactorily explained yet, neither from the taxonomic, nor from the biochemical point of view. For a longer period it was believed that the straightening is irreversible (Tomaselli 1997), but later Wang and Zhao (2005) proved that straight trichomes of *Arthrospira platensis* can revert their morphology to the usual helical structure.

Cultivation of *Arthrospira* began in France and Mexico in the 1970s (Durand-Chastel 1980; Shelef and Soeder 1980) using *A. platensis* and *A. maxima*. Ripley D. Fox did much

Fig. 26.3 *Arthrospira* production in volcanic crater Twyn Taung in Sagaing Province, Upper Myanmar (22°21′50.79″ N 95°01′28.31″ E)

to publicize the cyanobacterium in the next two decades, including his own work on integrated systems for village production (Fox 2001). Other researchers who helped to develop mass culture methods include Richmond and Vonshak (1978) on practical methods for developing large-scale cultivation in Israel and Soeder (1992) on raceway pond technology. The commercial large scale cultivation of *Arthrospira* as food and feed ingredient was established in the late 1970s in Thailand by Dainippon Ink & Chemicals, Japan, (DIC) and in the early 1980s by Proteus Coorporation in the USA, which was later incorporated into DIC. Today numerous companies are producing *Arthrospira* worldwide in an estimated output of over 5,000 t of dry weight per year (Lu et al. 2010; Rosello Sastre and Posten 2010). *Arthrospira* is cultured in constructed outdoor ponds in Africa, USA, Thailand, China, Taiwan, Myanmar and India (Chap. 25).

One of the oldest production facilities is in Calipatria, California, USA, maintaining a total of 300,000 m² pond surface in 2011 for the production of *Arthrospira* for food and feed. Production, which started in summer 1983 at "Earthrise Farms" is predominantly carried out in open raceway ponds each with an area of 1,000 to 5,000 m² and a depth of 0.15–0.3 m. A paddle-wheel mixes the suspension continuously, not exceeding velocities of 0.3 m s^{-1} in order to avoid shear stress and damage to the trichomes. The optimal biomass concentration for production lies between 0.4 and 0.5 g L^{-1}. Due to its location in southern California evaporation in the order of magnitude of several hundred m^3 day^{-1} for the whole plant has to be replenished by water from the Colorado River (Belay 1997). Raw water with a high calcium ion concentration leads to precipitation of calcium phosphate and probably iron phosphate, and hence a reduction in P and Fe available for the organism. Pretreatment of the water, CO_2 addition and removal of detritus are measures help to minimize problems associated with use of the river water. Due to the low temperatures during winter, production is restricted to April–October. There has been a tendency observed for increased coiling of trichomes throughout the cultivation period, resulting in a decrease in trichome length of about 34%. Higher temperatures as well as mechanical stress during harvesting seem to be responsible for the changed morphology (Belay 1997).

Another large production facility located in Kona, Hawaii (Cyanotech Corporation) has operated since 1984; in 2010 it had a total pond area of 116,000 m² and an average pond size of about 2,900 m² (Cysewski 2010). Here, consistent temperatures and sunlight allow production all the year. However, China has now become the world's largest *Arthrospira* producer. Part of the success is due to adaptation of the cultivation strategy in Inner Mongolia for growth in a much colder climate, yet one with high radiant energy during summer. A significant proportion of the produced biomass now comes from this region, where average temperatures of 6.4°C strongly reduce growth of mesophilic organisms. Thermo-proof greenhouses are placed over the raceway ponds in order to prevent growth inhibition or even culture deterioration. Under these conditions productivity is relatively low (5–9 g m^{-2} day^{-1}) and production is limited to about 5.5 months a year (Lu et al. 2010).

Besides many local or regional producers in India, South America and Africa a single mass producer from Myanmar is operating on the world market. The biomass is produced in natural crater (Fig. 26.3) lakes where the pH is high. The majority of the biomass is produced in the Twyn Taung crater, a lake of 200 ha area. Its salinity is 4 ppt and the pH at 9.5. It is operated for biomass production since 1988. In order to enhance growth combined nitrogen is replenished in regular intervals. The production capacity lies in the range of 200 t per year (Thein 2011). During summer month a maximum of 5 t per d is harvested from boats, while in 'off season' the production is much lower (below 1 t per d). In March, daily water temperatures range from 23–27°C.

Harvesting of the biomass is carried out via different, partially automated filtration techniques: inclined gravity screens, horizontal vibration sieves and vacuum filters are combined in order to dewater and desalinate the biomass to a solid content of 8–12%. The configuration of the different solid liquid separation steps needs to be tuned depending upon the amount of biomass that needs to be removed and upon the trichome size of *Arthrospira*. Vibro screens with a mesh size of about 100 μm are being used (Grobbelaar 2009a). The input of energy can be problematic for the cultures, since shear forces can lead to breakage of the trichomes. This results in reduced harvest efficiency, because small fragments pass the screens. In addition, bacterial contamination is increased due to the release of organic compounds by broken trichomes. In general, separation of solids from liquids requires more effort if cell densities are low.

Subsequently the biomass is dried and this may be done by technically easy procedures such as sun or oven drying or the more demanding use of drum and spray drying. The decision about the method largely depends on the investment capital available to the company. The resulting *Arthrospira* powder contains a residual moisture of 3–5%. It is certainly possible to obtain stable powders using low cost methods (Tiburcio et al. 2007). However, the conditions during drying influence the quality of the final products: hot temperatures or long drying times increase degradation of valuable ingredients such as carotenoids, enzymes and polyunsaturated fatty acids. Oliveira et al. (2010) found for both drying temperature and layer thickness a significant effect on the product quality of *Arthospira platensis* employing convective drying. Packaging is carried out directly after the drying process, preferably under vacuum and in non-transparent oxygen barrier bags rather than in polyethylene, in order to prevent oxidation of ingredients during storage (Gershwin and Belay 2007).

Beyond the nutritional value of the biomass, the numerous health benefits of *Arthrospira,* assessed by various studies, are of growing interest and are facilitating the market size. Amongst others anti-inflammatory, exhaust relief, immune system boosting, assisting in digestion and improvement of well-being have been claimed (Jensen et al. 2001). A therapeutic value in animal models and/or in humans was observed in the context of lowering hypertension, regulating hypercholesterolemia and hyperglycerolemia, enhancing the immune system, contributing to the stimulation of intestinal lactobacilli, reducing nephrotoxicity caused by heavy metals and drugs, protecting against radiation damages and being active against some cancer types, e.g. oral leukoplakia (Blinkova et al. 2001; Belay 2002; Gershwin and Belay 2007; Deng and Chow 2010). The enhancement of the immunity against different infections was often reported in pre-clinical studies (Capelli and Cysewski 2010). Those effects were connected to the enhanced production of antibodies and cytokines, as well as to the activation of macrophages, T and B cells (Blinkova et al. 2001). The cardiovascular benefits of *Arthrospira* are primarily resulting from its hypolipidemic, antioxidant, and antiinflammatory activities (Deng and Chow 2010).

High molecular weight polysaccharide preparations were isolated, e.g. “Immulina” from *A. platensis* that showed a high immunostimulatory activity, between 100 and 1,000 times more active for in vitro monocyte activation than polysaccharide preparations that are currently used clinically for cancer immunotherapy (Pugh et al. 2001). Moreover, in vitro a strong action against *Candida albicans* and tetanus toxoid was measured. In a human clinical trial the immune markers tumor necrosis factor alpha (TNF-α), interferon gamma (INF-γ) and interleukin-6 (IL-6) were significantly enhanced after administration of a high-molecular-weight polysaccharide extract from *Arthrospira platensis* (Løbner et al. 2008). Parages et al. (2012) investigated the immunostimulating effect of an acidic polysaccharide isolated and purified from a laboratory culture of *Arthrospira platensis*. The proinflammatory activity of the received fraction suggests that the polysaccharides could stimulate the immune response of the cells by inducing the production of the cytokine TNF-α in macrophages. The polysaccharide fractions, mainly sulfated, show anti-viral properties that are of great interest for the development of therapeutic drugs. Hernandez-Corona et al. (2002) detected also a high antiviral activity against HSV-2, in a hot water extract of *A. maxima*. Further purification of these fractions led to the identification of calcium-spirulan (Ca-Sp), a sulphated polysaccharide composed mainly of rhamnose, from *A. platensis* by Japanese researchers. It inhibited replication of HIV-1, human cytomegalovirus, measles, mumps, influenza A and HSV-1 (Hayashi et al. 1996). Its antiviral effect was found to be superior to that of dextrane sulfate against HIV-1 and HSV-1. The action is based on the selective inhibition of virus penetration to the host cells.

Sandau and Pulz (2009) also found a high activity of Ca-Sp against HSV-1, measured superior above Acycloguanosin, an active agent against HSV that is currently on the market.

Interestingly, the *in vitro* antiviral activity was already linked to the dietary consumption of *Arthrospira* by humans *in vivo*; the HIV infection rate in Chad is low compared to the rest of Africa, where *Arthrospira* is a traditional ingredient in the diet (Teas et al. 2004).

Ca-Sp was also found to be active against tumor invasion and metastasis of B16-BL6 melanoma cells by inhibiting the tumor invasion probably through the prevention of the adhesion and migration of tumor cells to laminin substrate and of the heparanase activity (Mishima et al. 1998).The invasion of carcinoma, melanoma and fibrosarcoma was inhibited by Ca-Sp (Capelli and Cysewski 2010). Water extracts of

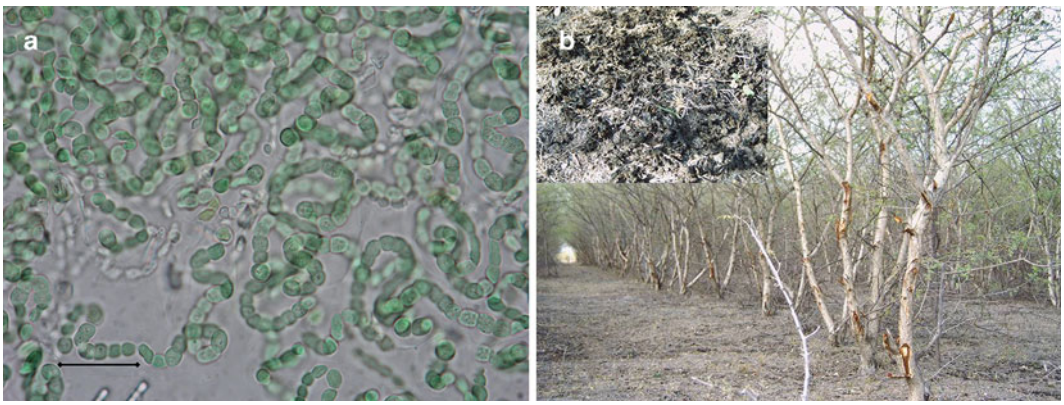

Fig. 26.4 (**a**) *Nostoc ellipsosporum* from liquid laboratory culture (SAG 1453-7), scale bar = 20 μm; (**b**) growth of *Nostoc flagelliforme* in its habitat on soil in Myanmar

Arthrospira have been reported to be cause regression of cancer progression in rodents (Grawish 2008; Akao et al. 2009; Grawish et al. 2010).

Anwer et al. (2012) reported the presence of insulin as a hypoglucemic agent in the range of 2 to 33 μg*g^{-1} within the biomass of 16 out of 23 investigated *Arthrospira* strains cultured under laboratory conditions. Its content was positively connected to the log phase of growth and influenced by the nitrate, phosophate, sulfate and bicarbonate concentration in the medium in *Arthrospira platensis*. The prebiotic effects of *A. platensis* biomass, both pure and in functional food application (biomass and aqueous extracts in processed in pasta, biscuits and others), were investigated on intestinal bacteria (Pulz and Gross 2004). An up to tenfold increase of growth rate of various lactobacilli was found, especially on *Lactobacillus acidophilus*. The measured effects were regarded as beneficial, although the components responsible and their mode of action have still not been explained satisfactorily.

The historical use of the biomass as food as well as safety studies imply that human consumption is generally safe. However, rare cases of side-effects in humans have been reported (Mazokopakis et al. 2008). The accumulation of heavy metals by the cells grown in open photobioreactors may be the highest risk, though this should be limited if there are stringent quality control measures. Moreover the produced biomass can contain alien cyanobacteria such as *Anabaena*, which may produce the neurotoxin anatoxin-A, so that neurological reactions can occur after consumption of *Arthrospira* (Grobbelaar 2003). Stringent quality control and maintenance measures will need to be applied in order to avoid damage of the industry.

The strong evidence that the intake of a few grams of *Arthrospira* (in the range of 2–13 g day^{-1}) leads to an array of therapeutic benefits will most certainly result in its still wider use as a nutraceutical food supplement worldwide. However, although the available data are many and coherent, further clinical research is needed to solidify the case for its use.

26.2.3 *Nostoc*

Nostoc colonies have been used as a food in Asia, especially China, for more than 2,000 years (Gao 1998; Qiu et al. 2002). Both their herbal and their pharmaceutical value contribute to their economic importance (Khaing 2004). *Nostoc commune*, *N. sphaeroides, N. verrucosum* and *N. flagelliforme* are the main species, with the last being probably the best known. Due to its appearance *N. flagelliforme* is called 'Fa cai' (hair vegetable) and grows on soil in China and Myanmar throughout the year (Fig. 26.4).

In Myanmar the organism reported as *N. verrucosum* grows attached to cliffs or on the soil, though in the latter case only during rainy season (Min Thein, personal communication 2011). (The original description of this species and almost all other subsequent reports are for flowing water, so it seems possible this may be another species.) Clouds have to prevent direct illumination for at least 5 days after the rain occurs to promote its growth. *N. sphaeroides* is collected from rice fields in China, where its colonies comprise dark green, subspherical colonies up to 25 mm diameter (Helblin et al. 2006). This is probably the same as the Ge-Xian-Mi reported by Qiu et al. (2002) from many parts of China, including Hefeng County, the location of their study. In this case recent agricultural changes, such as addition of fertilizer to the rice-fields, had led to a marked decrease in the *Nostoc* population, which in economic terms may not have been replaced by the increased rice yield.

In the natural habitat described by Danxiang et al. (2004), *N. flagelliforme* is about 0.5 m long, 0.2–1 mm in diameter and usually unbranched. In China the species grows in arid or semi-arid steppes of the west and north-west, where it usually occurs in the altitude range 980–2,800 m, often together with *N. commune* (Danxiang et al. 2004). There are also records for the species in dry regions of many other parts of the world, including Africa, Europe and USA. *N. flagelliforme* is physiologically adapted to both

drought and heat. Temperature extremes ranging from −29°C to 66°C have been reported from China (Gao 1998). The optimum temperature reported by Diao (1996) for growth was in the range of 15–25°C and for nitrogenase activity 21–28°C (Zhong et al. 1992); the heat tolerance is restricted to dry conditions (Danxiang et al. 2004). Among many protective mechanisms involved in desiccation tolerance are the presence of a high molecular weight extracellular polysaccharide that prevents phosphytidyl choline membrane fusion, and also the presence of a water stress protein (Hill et al. 1997). In order to understand the physiological processes during drought stress Liu et al. (2012) investigated genes of *Nostoc flagelliforme* exposed to sorbitol and reported a differential regulation of drought tolerance-associated genes providing an insight into molecular mechanisms connected to its drought adaptation. During rehydration colonies expand as water is taken up. Yoshimura et al. (2012) recently investigated the role of the extracellular polysaccharide from a terrestrial Nostoc sp. and linked its function to salinity tolerance. Under salt stress the amount of capsular polysaccharides increased up to 65% of dry weight, and a modified composition of monosaccharides was measured in *Nostoc* HK-01. Shi et al. (1992) reported on salinity tolerance, with values ranging from 0.05 to 0.9 M NaCl and maximum photosynthetic activity at 0.15 M. Light saturation occurred between 700 and 900 µmol photon m^{-2} s^{-1} (Zhong et al. 1992). As nitrogen sources both inorganic N (nitrate, nitrite and ammonium), organic N (amino acids) and N_2 can be utilized (Danxiang et al. 2004).

N. flagelliforme grows very slowly in its natural environment. Due to the reduced available area and an increasing market demand, attempts are being made to establish a cultivation technology. The first such attempts focused on solid media, due to its terrestrial habit (Cui 1983; Cheng and Cai 1988; Su et al. 2005). Cells divided 3–4 times in 10 days (25°C, low light conditions) when *N. flagelliforme* was cultured on solid medium (Cheng and Cai 1988), with growth in this case being enhanced with a soil solution extract obtained from its habitat. The maximum elongation rate was 43% in 12 days, with an average of about 20% for a similar period on a wheat field soil. Under aquatic conditions the best colonial development of the strain used by Gao and Ye (2003) occurred at 60 µmol photon m^{-2} s^{-1} and 25°C. On synthetic mats daily increases in dry weight of 0.6–6.1% have been observed (Qiu and Gao 2002); the procedure involved soaking the mats of *N. flagelliforme* with BG11-medium once or twice per day. A strategy to use liquid-grown cultures on sand bed materials has been developed by Chen et al. (2009, 2011). This approach can be used as a tool against soil erosion and desertification (Chap. 12).

A higher moisture content in the *N. flagelliforme* mats also encourages bacterial growth, which resulted in disintegration of the filaments after 7–10 days (Gao 1998). Pre-sterilizing filaments with 75% ethanol was effective against bacterial growth (Su et al. 2008), enhancing the elongation of filaments to 40% in 14 days at 30°C. Periodic desiccation seems to be important to prevent *N. flagelliforme* from being disintegrated by bacteria, indicating that drought is not simply an environmental stress, but of physiological and ecological significance (Gao and Ye 2003).

The few studies on the cultivation of *N. flagelliforme* in suspended culture have mostly been done in shaking flasks (Gao and Ye 2003; Liu and Chen 2003), but attempts at laboratory scale photobioreactors have been reported by Su et al. (2008). Trichomes from natural colonies were surface sterilized and then grown in a 20-L stirred photobioreactor with different agitation rates. Colony morphology, volumetric biomass productivity and EPS production were all affected by agitation speed. The highest volumetric biomass productivity (0.07 g L^{-1} day^{-1}) was reached at an impeller speed of 0.8 m s^{-1} and aeration rate of 0.8 vvm. Morphology in liquid culture changed from a compact colony to a thin slime formed around the cells. Another cultivation approach involving mixotrophic conditions showed comparable biomass productivity (0.04 g L^{-1} day^{-1}), but even higher biomass productivity of (0.23 g L^{-1} day^{-1}) (Yu et al. 2009). This yield was achieved at 25°C and continuous illumination using BG11-medium supplemened with 14 mM glucose; the initial pH was 8.0. Biomass productivity under both autotrophic and mixotrophic conditions was best at 60 µmol photon m^{-2} s^{-1}. This is the most efficient production method so far, but the work was done in shaking flasks (500 mL), so its scalability remains questionable. The results indicate that biomass yields are highly influenced by the nutrient supply, with the highest values under mixotrophic conditions, followed by heterotrophic and autotrophic growth, as it has been reported for several other cyanobacteria, including *Arthrospira* (Chen and Zhang 1997).

The physiological state of *Nostoc* colonies influences their carotenoid composition. Actively growing ones usually contain zeaxanthin, β-cryptoxanthin, myxoxanthophyll and β-carotene as primary carotenoids. However, the composition of desiccated cultures or ones exposed to UV is dominated by secondary carotenoids such as echinenone and canthaxanthin (Ehling-Schulz et al. 1997; Scherzinger and Al Babili 2008).

Early reports from China mention the use of *Nostoc* spp. to treat diarrhea, hypertension and hepatitis. More recent investigations have shown that a hot water extract from *N. flagelliforme* has anti-tumour activity, and an acid polysaccharide, Nostoflan, isolated from the *N. flagelliforme*, has anti-HSV-I activity (Kanekiyo et al. 2005; Kanekiyo et al. 2007). To the best of our knowledge no oral acute and subacute toxicity tests have been reported to establish the safety of *N. flagelliforme* for human consumption (Takenaka et al. 1998). Nevertheless the genus *Nostoc* is capable of producing

the neurotoxic amino acid β-N-methylamino-L-alanine. (BMAA) (Cox et al. 2005). Products of *N. commune* and *N. flagelliforme* traded in Hawaii, Switzerland and Peru have been investigated and some were found to contain BMAA (Johnson et al. 2008; Roney et al. 2009), a fact that needs to be considered as a safety concern.

The over-exploitation of *N. flagelliforme* in China (Qiu et al. 2002) show the need for further investigationson on the growth *Nostoc* spp. This is not only in order to establish and optimize a production process, but also to identify the conditions which favour toxin production with the aim of minimizing or avoiding any risk.

26.2.4 *Aphanizomenon flos-aquae*

In the early 1980s *Aphanizomenon flos-aquae* (AFA) was introduced to the US market as a health food supplement, and is therefore a relatively new food source, possessing a similar chemical composition as *Arthrospira*. The biomass was produced at Upper Klamath Lake, a shallow lake system in Oregon, USA. The production differs from *Arthrospira* in being harvested exclusively from a natural lake rather than from constructed ponds. According to Carmichael et al. (2000) blooms occur between late May and October or November with biomass concentrations of 3–50 mg L^{-1}. During this time a small bloom of *Anabaena flos-aquae* could occur (< 1% biomass), whereas *Microcystis aeruginosa* and *Coelosphaerium* (probably *Woronichinia*) appear in July and persist.

A laboratory study of two populations of *Aph. flos-aquae* from Japan showed that the organism could not grow below pH 7.1 and a temperature of 11°C, while growth tended to be suppressed under a light dark cycle of 10:14 (Yamamoto and Nakahara 2005).

Harvesting of the biomass from Lake Klamath is carried out via large harvesting nylon screens in different canals using the flow direction of the water and additionally pumping. One harvesting system is functioning via an aqueduct like system off lake the other one with on-lake barges. The biomass screens are guarded by debris screens in order to separate fish and floating material from the biomass and dewater the cyanobacterial biomass with a flow of up to 28.3 m^3 s^{-1} (Carmichael et al. 2000). A second vibrating screen concentrates the biomass to 1 g L^{-1}. Due to the variability of the environmental conditions that influence composition of the biomass, all settings need seasonal adjusting. Subsequently the biomass is pumped to a series of three slow- speed horizontal centrifuges, which remove sand etc. followed by a vertical high speed centrifuge that yields a product of 6–7% dry matter. Afterwards algae are chilled to 2°C and shock frozen in a flake freezer. Subsequently the biomass is freeze dried and the resulting product is used for the production on tablets or capsules.

Strict quality control procedures are necessary in order to avoid cyanobacterial toxins (Chap. 24), which have been reported for two strains of *Aph. flos-aquae* itself (Preußel et al. 2006; Wood et al. 2007). Assays such as mouse bioassays, ELISA and protein phosphatase inhibition assay (PPIA) are employed. The enzyme assays used are about 1,000 times more sensitive than the HPLC methods. Although the literature reports that *Aphanizomenon* can produce neurotoxins including saxitoxins and anatoxin-a, all tests on Klamath Lake cyanobacteria and the time of the review by Carmichael et al. (2000) failed to detect any cyanobacterial neurotoxins when examined by mouse bioassay, HPLC or mass spectrometry. The only cyanotoxin found in the phytoplankton during this testing period was microcystin from *Microcystis*, backed by the detection of microcystin syntase genes in health food supplements containing AFA by Saker et al. (2005). A risk assessment for the microcystin content of *Aph. flos-aquae* containing products has been carried out by Schaeffer et al. (1999), calculating 10 μg microcstin LR per g dietary supplement as safe based on a mouse feeding trial conducted in 1984.

Moreover anatoxin-a was found in *Aph. flos-aquae* strains isolated from toxic blooms of lakes in Finland (Rapala et al. 1993), who carried out a laboratory batch culture study. The toxin content of these strains was strongly influenced by growth conditions such as temperature, light intensity, nitrate and phosphate concentration; up to 19% of total toxins were released into the growth medium. Moreover PSP toxins have been identified in *Aph. flos-aquae* isolated from a river in Northern Portugal and of PSP toxins (neoSTX, dcSTX, STX and GTX5) in *Aphanizomenon* sp. (LMECYA31) after cultivation under laboratory conditions (Dias et al. 2002). One can summarize that of *Aph. flos-aquae* is capable of producing numerous toxins under several environmental conditions. Presumably strains differ in their toxicity and the ones assayed may differ from the one blooming at Klamath Lake. Although the production of *Aph. flos-aquae* at Klamath Lake benefits from the lack of costs during the growth stage, the numerous required toxin analyses reverse this advantage.

The quality assurance and related safety issues remain a problem for the marketing in other countries besides the USA. There is neither a food approval status for the *Aph. flos-aquae* biomass in the EU, nor a GRAS (generally regarded as safe) status in the USA. The detection of microcystins in commercial AFA products was published by German health protection officials in 2011, unsettling potential customers as well as biomass producers. The food industry insists on certified production processes for their products, which cannot be issued for the 'wild harvested' biomass. The label 'wild harvest' which had been a distinct marketing advantage in the health food market earlier (Carmichael et al. 2000) is no longer valid. In summary, we conclude that the ability of some *Aph. flos-aquae* strains to synthezise harmful toxins makes its consumption possibly

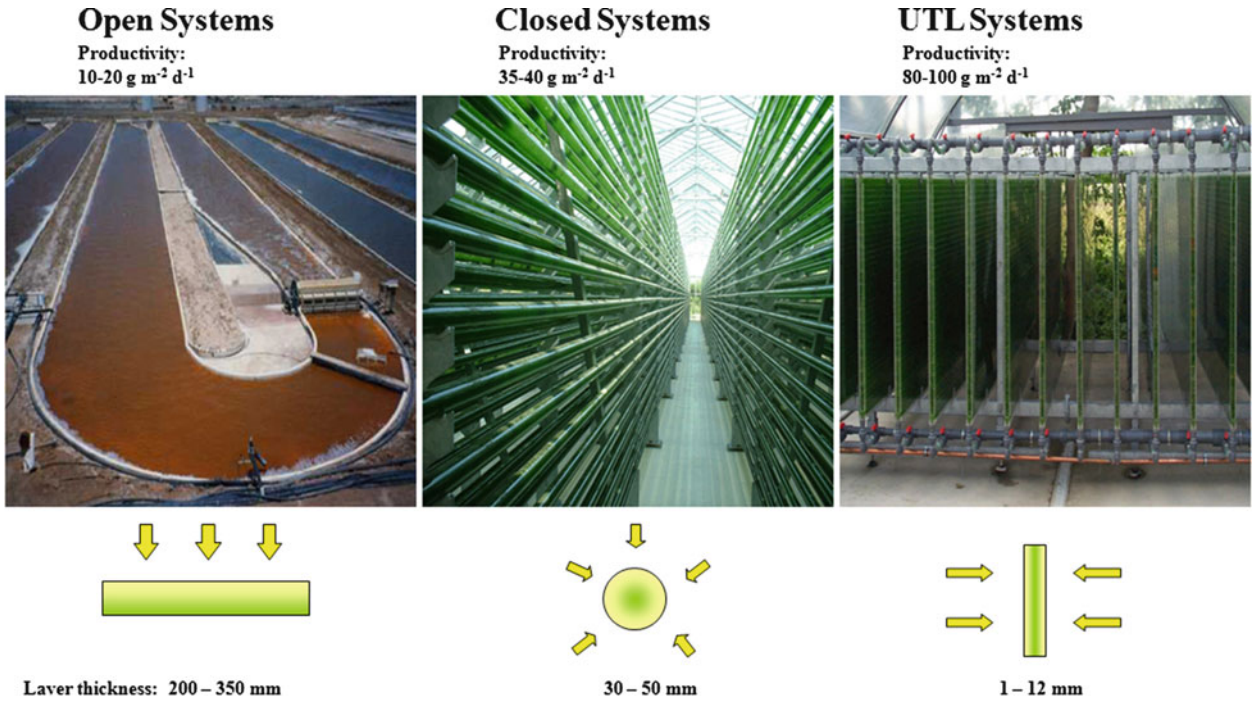

Fig. 26.5 Area productivity of various algae cultivation systems depending upon photobioreactors (*PBR*) geometry and layer thickness of photosynthetically active parts, *arrows* show light input : open system: raceway pond, closed system: tubular PBR, designed by Pulz (Pulz and Scheibenbogen 1998), ultrathin layer system: designed by Pulz for improved PBR performance

unsafe and thus conflicts with the initial purpose of a health food supplement.

Moreover the cause of *Aph flos-aquae* blooms in the lake is problematic. High P input is probably the main factor responsible for the blooms, which can lead to low oxygen concentrations when breakdown of the bloom occurs. In order to avoid hypoxic conditions, efforts are being undertaken by the State authorities to lower the load of total P within the next few years (Simon et al. 2009) to reduce the amount of bloom. In order to maintain the annual biomass output, open pond cultivation strategies would have to be applied. If successful, their application could enhance quality, yield and toxicological safety of the product.

26.3 Mass Culture of Cyanobacteria

In contrast to fermentation using heterotrophs, in photobioreactors the ability of phototrophic organisms to use photosynthesis as energy source is employed. The most important condition for the algal growth in the suspension is therefore the optimal provision with light, which cannot be stored in excess, like nutrients. The sufficient light supply can only be achieved by very thin layers of the culture suspension, given that by the algal growth and by the increased clouding of the suspension a rapidly decrease of the available light for the algal cells occurs. On the other hand only high cell densities in the culture assure high growth rates per volume unit. Just as with land plants, the culture area required to produce biomass plays a very important role.

Microalgae, including cyanobacteria, can be cultivated in open, closed or ultrathin layer systems (UTL) (Fig. 26.5). Open cultivation systems comprise natural or artificial ponds, raceway ponds, or so-called inclined surface systems. They present the classic method for the production of algal biomass and require large areas. If land utilization costs are low and climatic conditions favourable, the investment costs for sizes up to 100 ha are also relatively low. The greatest advantage of this approach to production is the low investment cost, which can be almost none if pond construction occurs in natural waters. Lake Chad is the best known natural system for *Arthrospira* production (Chap. 25). However, during the past decade a considerable *Arthrospira* production has also been developed in several alkaline crater lakes in central Myanmar. Natural and artificial ponds are generally used for the cultivation of marine, naturally predominant or extremophilic species, where there is a relatively low risk of contamination. Open pond systems dominate industrial scale algal biotechnology in general (Grobbelaar 2009b) and cyanobacterial biotechnology cultivating *Arthrospira* in particular, both in terms of annual output and their distribution worldwide.

The open ponds usually consist of cement or plastic basins, no deeper than 0.2–0.35 m, in order to maintain light conditions for optimal growth. The areas range from 25 to 5,000 m². The problem of stirring is of fundamental importance, because large amounts of energy are required to prevention

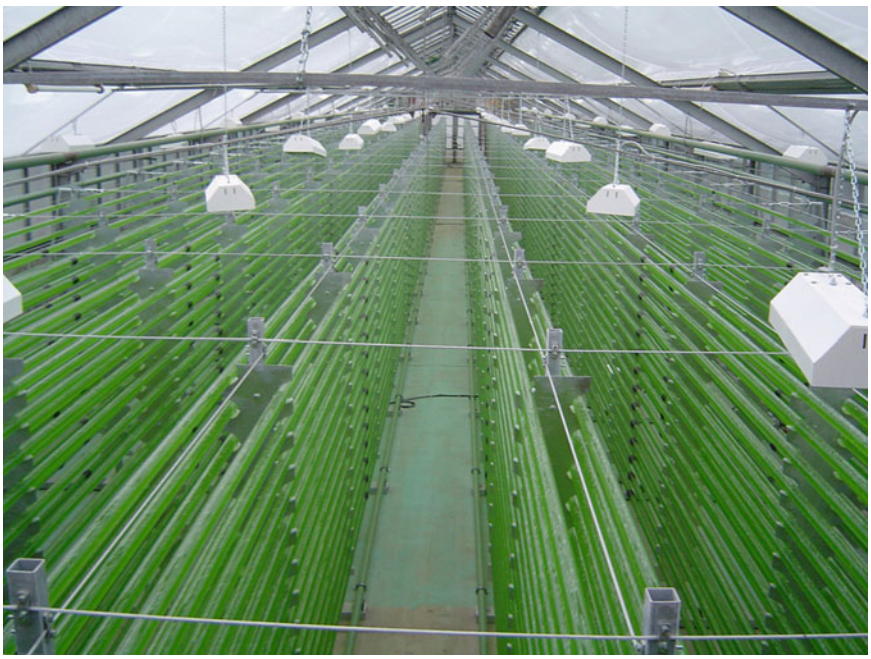

Fig. 26.6 A closed tubular photobioreactor plant at Salata GmbH, Ritschenhausen, Germany, installation by IGV GmbH, total volume 15,000 L, cultivation of *Oscillatoria* sp.

concentration gradients and algal sedimentation. Paddle wheels combined with aerating units incorporated into parallel, loop-like channels several km in total length is the common technique for the plants of several hectares, resulting in suspension velocities of 0.01–0.3 m s^{-1} and requiring relatively low operating expenses. The growth of biomass in raceway ponds is also dependent on the prevailing regional climate, the mean growth rates for *Arthrospira platensis* ranging from 5 to 20 g m^{-2} day^{-1}. Weather conditions like heavy rain, aridity and thunderstorms can influence the morphology and productivity of the cultures.

This cultivation method is restricted to tropical or subtropical climate zones, where the light input that is directly linked to the prevailing temperatures is sufficient for the ecological demands of the algae. Semiarid and arid climates mean less danger of flooding, but pose a higher water demand. Modifications have been introduced to permit growth in some mid-latitude maritime or continental locations, using greenhouses with foil in order to raise the temperature and therefore the production period per year (Lu et al. 2010). In such cases production is interrupted during the winter months, as occurs for *Arthrospira* production in China and France. Another advantage of the greenhouse is the possibility to introduce CO_2, increasing the CO_2 concentration in the culture suspension and thus the growth rate.

The low productivity and the vulnerability of open systems to contamination of various kinds has led to the development of closed reactors, in which photo-biological processes are less dependent on interfering environmental influences. Such closed reactors have a range of principal advantages: (1) *low CO_2-loss*; (2) *low water losses*; (3) *reduced contamination risk*; (4) optimal *temperature* regulation; (5) *controllable hydrodynamics*; (6) *reproducable cultivation conditions*; (7) considerable *flexibility regarding* environmental *influences*; (8) *low space requirements*. These photobioreactors allow introduction of light into the culture suspension by their light transparent reactor walls (tubes, plates); about 90% of the incident light can reach the cells in this system. In general the closed photobioreactor consists of photosynthetically active modules (glass or plastic tubes, or extruded profile plates), a compensation tank, distribution pipes and pumps. The reactor is characterized by its limited gas exchange with the surroundings. There is a relatively low contamination risk and the process is technically well controllable. In response to the various microalgal cultivation tasks and the specific requirements of particular species, many different reactor types have been developed, which are mainly used for research and development work. Extensive summaries of design principles are given by Ugwu et al. (2008) and Posten (2009).

Today the principal use of tube reactors is established for large-scale production. The largest European industrial algae production plant was built in Klötze, Saxony–Anhalt, Germany in 2001. The total production is realized in 20 plant sections, which work independently from each other. Each of these plant sections, which consist of a total volume of 35,000 L and a photosynthetic active tube surface of 3,500 m², is equipped with an independent control system. With such jointly connected bioreactor modules a maximum production capacity of 150 t per year can be produced on an area of 12,000 m^2. In 2005 a 85,000-L production plant was established in Ritschenhausen, Thuringia, Germany (Fig. 26.6). Here a single photobioreactor has a volume of 42,000 L and *Arthrospira* is produced with higher productivities than in the open systems. The biggest photobioreactor of the IGV Institut für Getreideverarbeitung GmbH was built with a total volume of 85,000 L as one module in Jerez, Spain, in 2011.

Fig. 26.7 Ultra-thin Layer PBR installation at APS Red Hawk Power Plant, Phoenix, AZ, USA; continous cultivation of a cyanobacterium for CO_2-sequestration using stack gas out of a gas fired power plant

Neither the open systems nor the described closed glass tube reactors meet the requirements of mass production of algal biomass for the production of bioenergy, such as biodiesel. Both systems fail to meet the efficiencies of biomass production and production costs required. Whereas in open systems productivities between 10 and 20 g m^{-2} day^{-1} can be reached, in the closed system described they are about 35–40 g m^{-2} day^{-1}. The biomass produced in the investment and operation cost intense closed photobioreactors, like the ones in Klötze and Ritschenhausen, is marketed successfully to the food and feed additive sectors. For bioenergy applications the production costs are too high.

The production principle has been developed further by IGV GmbH to produce a new design, the Ultra Thin Layer (UTL). This photobioreactor has been tested and showed positive results for functionality in a pilot plant in Arizona, USA (Fig. 26.7). The UTL-technology has led to an increase in productivity to 80–100 g m^{-2} day^{-1}. The yield for the UTL-system is 5 times higher than in an open system, mainly due to the high surface to volume ratio and improved gas exchange. This is the most productive photobioreactor reported so far (Posten 2009).

26.4 Present Uses

26.4.1 Food

In non -western civilizations algae have been used as food in the human diet for millennia. First records report about the consumption of macroalgae in coastal regions 6,000 years ago. Cyanobacteria, with the first records from *Arthrospira*, have been utilized by the Aztecs (Lake Texcoco, near Tenochtitlan) in Mexico by the Aztek population since 1300 AD (Pulz and Gross 2004) and in Africa (Lake Chad) even earlier (Abdulqader et al. 2000). *Arthrospira* is traded in Africa as *dihé* after collecting and sun drying (Ciferri 1983) and is consumed in soups at up to 60 g per meal (Delpeuch et al. 1975). In Mexico, the dried cake of *Arthrospira* called “tecuitlatl” was commonly eaten with maize, different cereals or in a sauce of tomatoes and spices. *Aphanothece sacrum* (Fujishiro et al. 2004), *Nostoc muscorum* and *N. commune* have been used as side dishes in Japan since ancient times (Lee 2008). Human consumption of *Nostoc* has been reported from Mongolia (China), Japan and Peru and also occurs in other Asian countries such as Myanmar (see Sect. 26.2.2). Dried and stir-fried *Nostoc* balls available on Asian markets are used mainly as soup ingredients. Since rising demands were driving the prices and massive collection of *N. flagelliforme* led to grassland degradation, desertification and social problems, all further collection, sale and exportation was banned by Chinese authorities (Roney et al. 2009). In order to follow the law an artificial *fa cai* was developed consisting of sepia-dyed starch noodles. However, similar material had been sold in Hong Kong long before that, even though many buyers at this time probably did not realize they were paying a lot of money for dyed starch (But et al. 2002). Other uses of cyanobacteria, such as *Aphanizomenon flos-aquae*, have been discussed above.

After the cyanobacterial biomass has been harvested and separated from the medium, it is processed to a sun- or

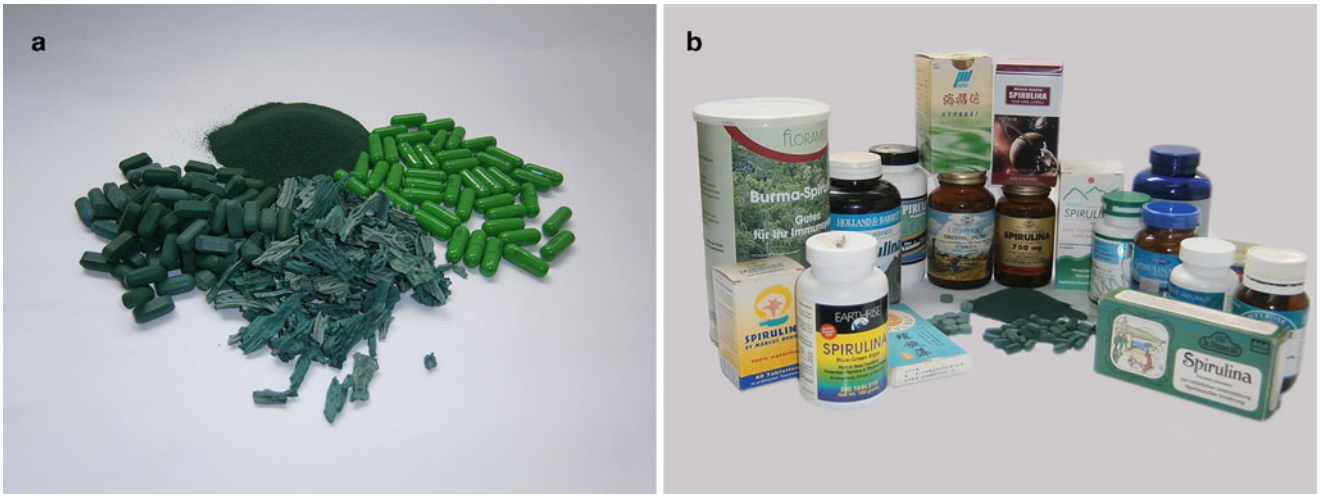

Fig. 26.8 (**a**) *Arthrospira platensis* processing forms (powder, flakes, capsules, tablets); (**b**) examples of *Arthrospira platensis* dietary supplement products

spray-dried powder of blue-green colour. In China it is almost always sold in normal shops and markets, but outside China mostly in health food stores. More than 95% of the annual microalgal biomass production is used for the manufacture of powders, tablets, capsules and pastilles (Fig. 26.8). Numerous combinations of microalgae or mixtures with other health foods can now be found on markets all over the world. The structure of the cell wall permits an easy digestion by humans and animals, which in turn accounts for the very high bioavailability of the valuable components of the biomass (Raja et al. 2008). This is an advantage over eukaryotic cells, which often need a separate cell disruption process due to their thick polysaccharide-containing cell walls hindering bioavailability.

Marketing and sales of algal biomass depend to a great extent on obtaining approval by the authorities. The market for dietary supplements is dominated by *Arthrospira*, the only cyanobacterial biomass that is food approved in the EU. *Spirulina* received GRAS in 1981 by the Food and Drug Administration of the USA, whereas *Aphanizomenon flos-aquae* has never been granted this status.

The idea to use microalgae as dietary supplements to cure malnutrition and in addition help close the protein gap in world nutrition due to its high protein content can be traced back to the 1970s. Important papers include (Soeder et al. 1971) and then ones by other pioneers of algal biotechnology such as (Richmond and Vonshak 1978) working with *Arthrospira*. Even today the consumption of microalgal biomass is restricted to a very few taxa, *Arthrospira*, *Nostoc* and *Aphanizomenon*, and *Chlorella*, *Dunaliella* and *Haematococcus* among the green algae. Cyanobacteria play the major role in terms of biomass for both traditional and current use. The exploitation of phototroph diversity was hampered by food safety regulations for human consumption for a long time. The successful authorization (following EC regulation 258/97) of microalgae or their extracts, such as the marine diatom *Odontella aurita* or the astaxanthin-containing *Haematococcus pluvialis* extract as novel food is a real advance for microalgal biotechnology. Facing the food regulations in the western countries there will soon be another problem – the fact that traditional collecting and trading of *Nostoc* and other microalgae for food purposes cannot keep up with the demands of an increasing population, particularly in Asia. The harvest is often exceeding the growth in natural habitats, exploitation of land decreases the production area and moreover the use of herbicides and pesticides in agriculture is negatively affecting the algal growth. The only promising solution for this challenge is the development of straightforward biotechnological production processes.

Studies promoting the use of *Arthrospira* as nutraceuticals are facilitating the development of extraction techniques in order to develop functional, new neutraceuticals. Therefore downstream processing techniques, such as supercritical fluid extraction (SFE) are optimized on high yields of valuable compounds, such as carotenoids, vitamins and fatty acids. For the purification of γ-linoleic acid from *A. platensis* two strategies are possible: column chromatography and urea crystallization of saturated fatty acids (Sajilata et al. 2008a) and supercritical carbon dioxide extraction (Sajilata et al. 2008b). A major advantage of CO_2-SFE is that the solvent is generally recognized as safe (GRAS), while a drawback is that the biomass needs to be dried prior to extraction. The extraction yields can be significantly enhanced using ethanol as an entrainer (Sajilata et al. 2008b). Mendiola et al. (2008) optimized extraction of tocopherol from *A. platensis*, reaching final contents of 2.9% in the extract.

Currently most products launched to serve the health food market are supplied as tablets and powder. Nevertheless, functional food or nutraceuticals produced with microalgal biomass or algal extracts are sensorically much more convenient than algal powders. In Germany, food production and distribution companies have started serious activities to market functional food with microalgae and cyanobacteria. Examples are pasta, bread, yogurt, sweets and soft drinks. Similar developments can be observed, for example, in Japan, USA, China and Thailand. New product developments will combine health benefits with attractiveness to consumers and create a stable market in the future. The market of functional foods is believed to be the most dynamic sector in the food industry and could constitute up to 20% of the whole food market within the next few years.

26.4.2 Special Ingredients – Phycobiliproteins

In addition to chlorophyll a, as the primary photosynthetic pigment, microalgae contain a multitude of pigments which are associated with light harvesting. Over 100 different carotenoids are synthesized by microalgae of all divisions and classes (Liaaen-Jensen and Egeland 1999), but the biosynthesis of phycobiliproteins is restricted to cyanobacteria, rhodophytes and cryptophytes (Eriksen 2008). They are divided according to their absorption characteristics to the main classes phycocyanin (PC, λ_{max} 610–620 nm), allophycocyanin (APC, λ_{max} 650–655 nm) and phycoerythrin (PE, λ_{max} 540–570 nm) (Bermejo Román et al. 2002). Phycobiliproteins are multi-chain proteins consisting of apo-proteins and phycobilins (linear tetrapyrrols) covalently bound to specific cystein residues of the protein. The three dimensional structure of c-phycocyanin (C-PC), with minor species-dependent variations has been elucidated in various cyanobacteria (Dobler et al. 1972; Stec et al. 1999; Padyana et al. 2001; Contreras-Martel et al. 2007). In cyanobacteria phycobiliproteins are incorporated into phycobilisomes located in the outer thylakoid membrane and improve the efficiency of light energy utilization by broadening the absorption spectrum of light, transferring the excitation energy by radiation less processes to the reaction centres (Eriksen 2008). In addition to their role in light absorption, they can serve also as a N store that is mobilized in case of N depletion in their environment (Boussiba and Richmond 1980).

Phycobiliproteins are water-soluble, scavenge free radicals and are strongly fluorescent. Among several potentially useful effects of phycocyanins which have been shown are antioxidant and anti-inflammatory properties (Benedetti et al. 2004). C-PC is a selective inhibitor of cytochrome oxidase 2, resulting in hepatoprotective action and a reducion in leucotriene B4 levels, a reaction responsible for its anti-inflammatory properties. It has also shown to have therapeutic value by immunomodulating activity and anticancer activity (Rasool and Sabina 2009).

Table 26.2 Commercial application of phycobiliproteins

Industrial sector	Product/application
Food industry	Candy, chewing gums, ice creams, dairy products, soft drinks, wasabi
Cosmetic industry	Eye shadow, eye liner, lip sticks
Analytics	Flow cytometry, fluorescent activated cell sorting, fluorescence immunoassay and fluorescence microscopy

An important application for phycobiliproteins is their use as natural dyes in foods and cosmetics, thus replacing synthetic colourants that are often toxic, carcinogenic or otherwise unsafe (Bermejo Román et al. 2002). The phycocyanin extract produced from *Arthrospira* is market under the name 'lina blue' and used for different products in food and cosmetic industry (Table 26.2). In the analytical sector both PC and PE are being used as fluorescent tags.

The industrial production of phycocyanin (C-PC) is based on open pond cultures of *Arthrospira platensis*, that of phycoerythrin on *Porphyridium cruentum* (Bermejo Román et al. 2002). Although C-PC is presently extracted from *Arthrospira*, other cyanobacteria have been used for downstreaming method development as well (Table 26.3). The establishment of C-PC production with *Arthrospira* is mainly due to its availability from outdoor cultivation in Asia. The C-PC yield depends mainly on volumetric biomass productivity and the C-PC content of the cells. Table 26.4 summarizes the organisms, their biomass productivity in different photobioreactor types used as well as the C-PC content in the biomass.

Although autotrophic, mixotrophic and heterotrophic cultivation have been investigated for the production of *Arthrospira* (Chojnacka and Noworyta 2004), the highest productivity was achieved under mixotrophic conditions. Both biomass and pigment production was enhanced in the range of 1.5–2-fold compared to photoautotrophic growth (Marquez et al. 1995), but this has not been established in commercial cultivation, mainly due to quality issues. The total bacterial count cannot be controlled in open systems, as organic C sources lead to a much faster growth of obligate heterotrophs. Comparisons of heterotrophic growth of various axenic *Arthospira* strains have identified glucose and fructose as suitable C sources in concentrations of 20 mM (Chojnacka and Noworyta 2004; Mühling et al. 2005a). Both growth rate and C-PC productivity are lower than with autotrophic and mixotrophic growth (Eriksen 2008) and greater effort is required for sterilization of culture equipment. Heterotrophic production of C-PC with the rhodophyte *Galdieria sulphuraria* may have greater potential, since biomass productivity of 50 g L^{-1} day^{-1} and C-PC productivity of 0.9 g L^{-1} day^{-1} have been measured

Table 26.3 Downstream processing methods for purification of phycocyanin from cyanobacteria (Based on Eriksen 2008)

Cell disruption (CD): Liquid nitrogen (CD1), sonication (CD2), freeze thaw cycles (CD3), osmotic shock (CD4), enzymatic treatment (CD5)
Extraction (E): Phosphate buffer (E1), aqueous two phase extraction (E2), aqueous extraction (E3)
Precipitation (P): $(NH_4)_2SO_4$ (P1), rivanol (P2)
Impurity adsorption (A): Activated charcoal (A1), chitosan (A2)
Concentration (C): Dialysis (C1), ultrafiltration (C2)
Chromatography (CH): Anion exchange chromatography (CH1), gel filtration chromatography (CH2), expanded bed chromatography (CH3), hydroxyapatite chromatography (CH4), hydrophobic interaction chromatography (CH5)

Species	Purification method	Purity C-PC	Yield	References
Arthrospira platensis	E2 (PEG 4000/potassium phosphate)	4.05	85%	Patil and Raghavarao (2007)
	P1, C4, CH1	4.15	–	Boussiba and Richmond (1979)
	CD3, P1, C1, CH1 (DEAE-Sepharose, fast flow)	5.59	67%	Yan et al. (2011)
	P1, CH1, CH2	5.06	–	Zhang and Chen (1999)
	E2, CH1	6.69	–	Patil et al. (2006)
	CD1, E1, P1, C1, CH2	4.98	–	Bhaskar et al. (2005)
	CD2, E1, A1 & A2, C2, CH1 (DEAE Sephadex A-25)	4.3	42.3%	Liao et al. (2011)
	CH3 (Phenyl-Sepharose)	2.87	3.1%	Niu et al. (2007)
	CH1	3.2	0.77%	
	CH4	3.2	0.45%	
Arthrospira maxima	A1, C2	0.74	14.1%	Herrera et al. (1989)
	A1, P1, C1, CH2 (G200), CH3 (DEAE 100)	3.91	3.6%	
	E2, C2, P1	3.8	29.5%	Rito Palomares et al. (2001)
Arthrospira fusiformis	P1 & P2, CH2 (Sephadex G-25), P2	4.3	46%[a]	Minkova et al. (2003)
Aphanizomenon flos-aquae	P1, CH4	4.78	–	Benedetti et al. (2006)
Microcystis aeruginosa	CD3, E1, CH1 (DEAE cellulose)	–	–	Padgett and Krogmann (1987)
Synechocystis aquatilis	CD4, CH1 (DEAE-cellulose)	–	69%	Ramos et al. (2011)
Synechococcus sp. IO9201	CD3, CH5, CH1 (Q-sepharose)	4.85	–	Abalde et al. (1998)
Phormidium fragile	CD1, P1, CH5	4.52	62%[a]	Soni et al. (2008)
Phormidium ceylanicum	CD3, C2, CH1	4.15	63.5%[a]	Singh et al. (2009)
Oscillatoria quadripunctulata	CD3, P1, CH2 (Sephadex G-150), CH1 (DEAE cellulose)	3.31	68%[a]	Soni et al. (2006)
Calothrix sp.	CD5 (lysozyme), CH1 (Q-Sepharose, fast-flow), CH5	–	–	Santiago-Santos (2004)

[a]Referring to the crude extract

Table 26.4 Overview of biomass and phycocyanin productivity in two cyanobacteria and the rhodophyte *Galdieria* sulphuraria according to production and cultivation conditions (Based on Eriksen 2008)

Species	PBR	Volume (L)	Conditions	P_x (g L^{-1} d^{-1})	P_{C-PC} (% of dm)	References
A. platensis	Raceway	135,000	Autotrophic	0.05	6.1	Jiménez et al. (2003)
	Raceway	300	Autotrophic	0.18	6.7	Pushparaj et al. (1997)
	Tubular	11	Autotrophic, ID 0.01 m	1.32	7.0	Carlozzi (2003)
	Bubble column	12	Autotrophic, ID 0.47 m	1.05	7.0	Zitelli et al. (1996)
	Fermentor	2.5	Mixotrophic, fed batch	0.82	12.5	Chen and Zhang (1997)
	Fermentor	2.5	Autotroph		13.5	Chen and Zhang (1997)
Anabaena sp.	Raceway	300	Autotroph	0.24	5.6	Moreno et al. (2003)
Galdieria sulphuraria	Fermentor	2.5	Heterotroph	50	1.8	Graverholt and Eriksen (2007)

P_x volumetric biomass productivity, ID inner diameter

(Graverholt and Eriksen 2007); however, the absolute C-PC content is only about one-third that of *Arthrospira*.

The C-PC content in *A. platensis* ranges from 6.1% to 13.5% biomass, it being easier to obtain high values in small-scale laboratory fermentors because adequate light supply can be realized. Glucose has no or only minor effects on the C-PC content of *Arthrospira* (Chen et al. 1996; Eriksen 2008). The light supply can be considered the most critical factor for biomass productivity in autotrophic cultures (Pulz and Scheibenbogen 1998). In industrial scale raceway ponds an average value of 6% C-PC is reached with a biomass productivity of 14–23.5 g m^{-2} day^{-1}

(Pushparaj et al. 1997; Jiménez et al. 2003; Moreno et al. 2003). As soon as closed photobioreactors are applied for biomass production, the lower light path and higher cell densities result in higher productivities of biomass and C-PC. Volumetric productivity values above 1 g L^{-1} day^{-1} have been reported for small-scale photobioreactors, corresponding to 47.7 g m^{-2} day^{-1} (Carlozzi 2003). The absolute C-PC content has not been found to be significantly higher in closed photobioreactors, although its productivity was enhanced by a factor nine compared to value reported by Pushparaj et al. (1997). However, no data on C-PC production in large-scale closed photobioreactors are available, probably because of the high production costs, that are not compensated by the higher productivity. The C-PC content in *Anabaena* sp. was about 5.6%, and thus in the same range as *Arthrospira* (Moreno et al. 2003), whereas that of *Aphanizomenon flos-aquae* can be as high as 15% dry matter (Benedetti et al. 2004).

Several researchers have investigated the possibility of recombinant production of C-PC in *E. coli* (Cai et al. 2001; Tooley et al. 2001; Ge et al. 2005; Guan et al. 2007). The work is being hampered by the fact that the α- and β- chain of those multi-chain proteins needs to be expressed simultaneously together with the synthesis and correct insertion of the chromophores (Eriksen 2008).

For the downstream processing of the C-PC, methods with varying purities and yields of the pigment have been developed for different *Arthrospira* spp. (Boussiba and Richmond 1979; Abalde et al. 1998; Sarada et al. 1999; Zhang and Chen 1999; Rito Palomares et al. 2001; Bermejo Román et al. 2002; Doke Jr 2005; Niu et al. 2007; Oliveira et al. 2008; Patil et al. 2008; Soni et al. 2008; Ramos et al. 2011), *Aphanizomenon flos-aquae* (Benedetti et al. 2006), *Microcystis aeruginosa* (Padgett and Krogmann 1987), *Synechococcus* (Abalde et al. 1998), *Phormidium fragile* (Soni et al. 2008), *Phormidium ceylanicum* (Singh et al. 2009), *Calothrix* (Santiago-Santos 2004), *Oscillatoria quadripunctulata* (Soni et al. 2006), *Synechocystis aquatilis* (Ramos et al. 2011) and the rhodophyte *Porphyridium cruentum* (Bermejo Román et al. 2002).

Differences in the methods are needed because of the differing morphology of the various organisms, such as the structure of cell walls and membranes; no standard technique exists (Sekar and Chandramohan 2008). For high pigments yields, the cell wall needs to be broken prior to PC extraction. In the case of wet biomass processing, freezing-thawing-cycles have been found to be very effective for the rhodophyte *P. cruentum* (Abalde et al. 1998) and this method has been employed successfully for *Arthrospira* (Zhang and Chen 1999) and *Phormidium* (Soni et al. 2008). For cell disruption, mechanical methods such as ball mills, high pressure homogenization, French press, liquid nitrogen, mortars or sonication are used (Eriksen 2008). The application of diluted buffers for an osmotic shock or the use of enzymes such as lysozyme has also been reported for the extraction of phycocyanins (Boussiba and Richmond 1979; Sekar and Chandramohan 2008). Unfortunately many of the methods used in the laboratory are non-scalable (Bermejo Román et al. 2002). If dried biomass is extracted, it has been reported that a high drying temperature during the processes (flow drying, spray drying, sun drying and oven drying) are responsible for a significant yield reduction of C-PC (up to 50%) in *Arthrospira* (Sarada et al. 1999; Doke Jr 2005; Oliveira et al. 2008). After cell disruption, purification of the C-PC is done, usually in a two-step process involving extraction and purification of the raw extracts. Although different methodologies have been proposed for purifying phycobiliproteins, only some of them are useful for scale-up. The extraction from phycobilisomes and precipitation is mainly carried out by a $(NH_4)_2SO_4$ solution (overnight), whereas high ionic strength (0.5 M) leaves the phycobilisomes intact. Another frequently used extraction method is the resuspending of the biomass in phosphate buffer (0.1 M, pH = 7) and incubation at 4°C for 24 h (Doke Jr 2005). C-PC is thereby stable in the range of pH 5–7.5 at temperatures of 9–40°C, temperatures of above 40°C led to instability (Sarada et al. 1999). Another approach to C-PC purification was introduced by Rito Palomares et al. (2001), an aqueous two phase system (ATPS) using polymers (polyethylene glycol) and salts. This method in combination with ion exchange chromatography resulted in the highest C-PC purity reported so far (Patil et al. 2006) (Fig. 26.4).

Typically, phycocyanins are purified after extraction by a combination of several chromatographic techniques such as anion-exchange chromatography (Bermejo Román et al. 2002; Liao et al. 2011; Yan et al. 2011) with a pH gradient elution, hydrophobic interaction chromatography (Soni et al. 2008), gel filtration chromatography, column chromatography with hydroxyapatite (Benedetti et al. 2006), expanded bed adsorption chromatography (Ramos et al. 2011), as well as combinations of these (Abalde et al. 1998). Bermejo Román et al. (2002) developed a one-step scalable chromatographic method that provides B-PE solutions in hexameric aggregation state, as well as pure fractions of R-PC from *P. cruentum*. Dehydration is carried out by freeze drying, the gentlest method available. Liao et al. (2011) applied an additional adsorption step with chitosan and activated charcoal in order to remove impurities, which is cheap and effective and yields food grade purity.

The purity of the phycocyanins is expressed as ratios of A_{620}/A_{280} for C-PC and A_{650}/A_{280} for APC, respectively, while absorption at 650 or 620 nm accounts for the phycocyanobilin content which is specific for the protein, and the absorption at 280 nm, which represents all aromatic amino acid residues of the proteins. Throughout the purification methods A_{620}/A_{280} values between 0.7 (Herrera et al. 1989) and 6.69 have been recorded (Patil et al. 2006). A ratio greater than 0.7 is

recognized as food grade quality, while A_{620}/A_{280} of 3.9 is considered reaction grade and A_{620}/A_{280} of above 4.0 is analytical grade (Herrera et al. 1989). The overall yields of the downstream process vary with the method applied; being about 46% based in crude extract (Minkova et al. 2003) and go up to 85% total yield as reported by Patil and Raghavarao (2007).

In the development of the purification methods the focus is clearly laid on methods that comprise one chromatographic step; furthermore they need to be scalable, inexpensive and time saving. Both purity and yield serve as quality parameters.

Depending upon the purity and application required, the price for phycobiliproteins ranges between US$ 3 and 25 per mg for the pigment and up to US$ 1,500 per mg for antibody-complexes (Spolaore et al. 2006). Currently, numerous companies are producing PC, while 297 patents have been found by Sekar and Chandramohan (2008) in this connection. This documents the high degree of commercialization of cyanobacterial biotechnology in this field. Development will certainly go beyond applications in diagnostics and photodynamic therapy and extend to cosmetics, nutrition and pharmacy.

26.4.3 Animal Feed

Survival, growth, development, productivity and fertility of animals are basically determined by their health. Feed quality is the most important exogenous factor influencing this, especially in connection with intensive breeding conditions and the trend to reduce or to avoid antibiotics. The feeding trials of the past employing algal biomass were dominated by the use of high proportions of the common feed (up to 50%), aiming to replace the raw protein source. Now that research results have shown that very small amounts of microalgal biomass can have a positive effect on the physiology of some animals, lower amounts (0.1–10%) of *Arthrospira* are being used. Besides the positive effects of vitamins, minerals and PUFAs, an unspecific immune response and boosting of the immune system of animals has been observed and are considered to contribute to the positive results (Khan et al. 2005). The enhancement of growth, fertility, survival rate, live weight, feed conversion efficiency, resistance to bacterial and viral infections and enhanced colour have been reported in chickens, buffalos, prawns, salmon, carp and tilapia (Belay et al. 1996).

Arthrospira has proved very effective in the poultry industry for the colouration of egg yolk (Vonshak 1997a), reaching a maximum after 7 days diet. A content of 21% *Arthrospira* in the diet led to the colour score being 37% higher than indigenous eggs and 2.6 times more effective than dehydrated berseem (*Trifolium alexandrinum*) meal and 1.9 times more than 40% yellow maize. At all used proportions *Arthrospira*-fed birds produced egg yolks with a deeper colour than the two conventional carotenoid sources, clover and maize. The investigation of different processing methodologies in the preparation of quail feed revealed that freeze drying of the biomass is preferable to an extrusion process, since the latter degrades the carotenoid content of the cyanobacterial biomass by heat. Due to high processing costs freeze drying is not dominating feed production. The carotenoid content in the extruded *Arthrospira*-feed was also influenced by the raw material used and were higher for cassava than for corn or barley products (Ross et al. 1994). Sufficient colour scores of quail eggs were obtained with comparably low *Arthrospira* contents (0.5–4%) with no adverse effects on egg production, egg weight, final body weight or mortality. The enhancement of meat colour in muscle of broiler chickens with the use of 40 g kg^{-1} *Arthrospira* in the diet was positively correlated with the zeaxanthin content in the flesh (Toyomizu et al. 2001).

There are a number of reports of increased animal production in addition to improved quality. Supplemention of corn-based diet for hens with 1% *A. platensis* (1 g biomass per hen per day) led to an average increase of 51.7% in eggs laid over a trial period of 30 days (Fig. 26.9) compared to the control group (Fig. 26.9) (Storandt et al. 2000), whereas the egg weight increased by 2.4%. Moreover the firmness of the eggshell, the appearance of the plumage and general health were all influenced positively. The positive effect on egg productivity was still measurable in the subsequent period, when feeding was carried out without *A. platensis* addition. After another 10 days the number of produced eggs was identical between the cyanobacterial-fed group and the control. In a feeding trial on broiler hens a diet containing 0.1% *A. platensis* was shown to result in increased nutrient uptake and final slaughter weight (Pulz et al. 2008). The reduced percentage of *A. platensis* in the diet compared to previous work is an important cost factor for the farmer; economic analysis has shown that the increased slaughter weight pays for the increased feeding costs.

Aquaculture holds a specific position in the use of algal biomass as feed, since algae are the basis of the natural food chain in almost all aquatic systems. Beside the direct nutritional use for molluscs, zooplankton, crustaceans and fish larvae, they are being used as an addition for the enhancement of colouration, growth and immunity. More than 40 species of microalgae are being used in aquaculture worldwide depending on the special requirements for production. Key features needed are adequate cell size for ingestion by the particular animal, the absence of toxins and the nutritional profile, including ω3 fatty acids. Marine aquaculture is an important economic sector worldwide with a predicted growth trend of 8% p.a., which will lead to an increase in growth systems using artificial ponds. As feed production contributes a major cost input in aquacultural production, biotechnological production of algae is increasingly the focus of interest for

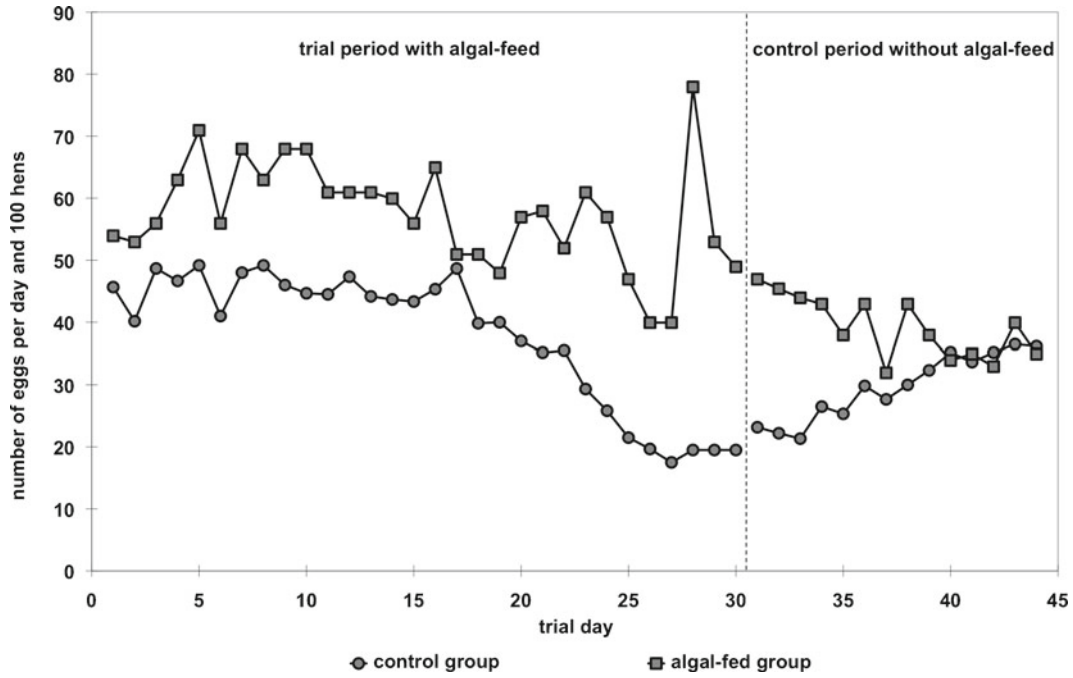

Fig. 26.9 Feeding trial for the investigation of the effect of 1% *Arthrospira* diet on the egg-laying productivity of laying hens in a free range breeding company in Brandenburg, Germany, compared to laying hen feed over a period of 30 days (*Arthrospira* uptake of 1 g per hen per day)

aquaculturists. The worldwide production of long chain polyunsaturated fatty acid containing eukaryotic algae, such as *Nannochloropsis*, *Tetraselmis* and *Isochrysis*, was estimated to be 1,000 t (Muller Feuga et al. 2003). Diverse, mostly technically inadequate equipment is used, resulting in low quality biomass, low yields and a high cost level. Cost estimates for microalgal products in the aquaculture sector normally range between US$ 50 and 200 per kg dry weight.

Among cyanobacteria *Arthrospira* is currently mainly used for aquaculture feed (Muller Feuga 2004), with most experience in the Japanese region. Its benefits have been investigated by several authors. El-Sayed (1994) reported that silver seabream utilized *Arthrospira* biomass more efficiently than either soy bean meal and chicken offal meal. The examination of *A. platensis* in the diet of tilapia (*Oreochromis mossambicus*) showed that it can replace up to 40% of the fish meal protein in tilapia diets (Olvera Novoa et al. 1998). Another species of tilapia (*O. niloticus*) fed solely on raw *Arthrospira* maintained its normal reproductive performance (fertilization rate, hatching rate of the fertilized eggs, survival time of larvae) throughout three generations (Lu and Takeuchi 2004). Other cyanobacteria have also been tested for their suitability as the sole source of nutrition. Cultured *Phormidium valderianum* was used successfully for tilapia production in India (Thajuddin and Subramanian 2005). The utilized strain tolerated high salinities and the biomass was incorporated into feed pellets in order to reduce handling at the production site. In view of the need for water quality control in aquaculture, *Arthrospira* has been successfully co-cultured with black tiger shrimp (*Penaeus monodon*), resulting in reduced N concentrations in the tanks and enhanced shrimp survival rate (Chuntapa et al. 2003).

Apart from feeding larvae and zooplankton the addition of *Arthrospira* to common fish feed compositions seems to be a promising strategy. Initially, the colour-enhancing effects of *Arthrospira* biomass were exploited for ornamental fish (Benemann 1992) and crucian carp (Min et al. 1999). The inclusion of *Arthrospira* into the diet of pond-reared prawn (*Fenneropenaeus indicus*) eliminated the pigment deficiency syndrome (PDS) at a level of 30 g kg^{-1} in the diet after a 4-week period (Regunathan and Wesley 2006). This confirmed the bioavailability of carotenoids from *Arthrospira* for shrimp broodstock and its regular use in the diet was recommended to avoid carotenoid deficiency-related problems in shrimp hatcheries.

With increasing use of *Arthrospira*, questions of feed utilization and health status in dense aquacultural fish populations became more important. Therefore, the immunomodulatory effects of *A. platensis* have been investigated by its inclusion in the diet of the carp *Cyprinus carpio*. For instance, immunostimulant effects were demonstrated by Watanuki et al. (2006). In an earlier study by Schreckenbach et al. (2001) both the unspecific cellular immune defense and the humoral specific immune immune response of the carp population were enhanced. The content of *Arthrospira* in the diet and the processing form of the biomass were identified as the main factors influencing how effective the response

Table 26.5 Examples of active substances from algae (utilized and potential sources) in marketed and potential cosmetic products (Modified after Sandau 2010)

Cosmetic activities	Active substances	Algae division	Utilized algae/potential sources
UV protection	Phlorotannins	Phaeophyta	*Ascophyllum nodosum*
	Colorless carotenoids	Chlorophyta	*Dunaliella salina*
	Mycosporin-like amino acids	Cyanobacteria	*Anabaena, Oscillatoria, Nostoc commune, Scytonema*
		Rhodophyta	*Porphyra umbilicalis*
	Photolyase	Chlorophyta	*Dunaliella salina*
	Scytonemin	Cyanobacteria	*Scytonema*
Skin protection	Radical scavenger: tocopherols, superoxid dismutase, polyphenols, β-carotene, carotenoids	Chlorophyta	*Dunaliella salina* *Haematococcus pluvialis*
	Phytohormones: auxins, gibberellins, cytokinins, abscisic acid and betaines	Phaeophyta	*Ascophyllum nodosum* *Stypocaulon scoparium*
Hydration/moisturizers skin protection	Polysaccharides, mucopolysaccharides, sulphated polysaccharides	Eustigmatophyta Rhodophyta Cyanobacteria	*Nannochloropsis oculata* *Porphyridium cruentum* *Arthrospira platensis*
Skin smoothing/skin regeneration	Essential amino acids	Cyanobacteria Chlorophyta	*Arthrospira platensis* *Chlorella vulgaris*
	Polyunsaturated fatty acids	Eustigmatophyta Rhodophyta	*Nannochloropsis oculata* *Porphyra umbilicalis*
Skin lightening/ skin whitening	Phlorotannins, phloroglucinol and its oligomers	Phaeophyta	*Ascophyllum nodosum* *Undaria pinnatifida*
Stimulating collagen synthesis	Low molecular weight fucans	Phaeophyta	*Fucus vesiculosus*

was. A similar immunomodulatory effect was reported for tilapia (*Oreochromis nilotocus*), but in this case correlated with the additional β-1,3-glucan content in an *Arthrospira*-containing diet (Cain et al. 2003).

In terms of cost effectiveness the immune enhancement and antibacterial and antiviral activities are much more desirable than the use of the biomass in high proportions as a partial substitute for higher plant or animal proteins. *Arthrospira* can help protect against the many pathogens which diminish the yields in aquaculture and agriculture industry. The use of antibiotics to control pathogens is costly and has undesirable health consequences for consumers. Today more studies on its immunomodulatory, antiviral and anti-cancer effects on various animals are available than on humans. Supplementation with *Arthrospira* may offer an alternative to common strategies relying on chemicals. Nevertheless the potential for incorporating cyanobacteria in feed is not utilized today. To the best of our knowledge not more than 1% of the worldwide produced biomass is utilized for different feed applications.

26.4.4 Cosmetics

With an aging world population and increasing per capita income, we see a growing market share for cosmetics in general and for anti-aging products in particular, especially in Europe, the USA and Asia. Along with their valuable nutritional ingredients many algae contain active dermogenic substances. Currently numerous products containing cyanobacterial extracts have been formulated and are being marketed, such Protulines® and Aquaflor®, both of which contain *Arthrospira* extracts with proven moisturizing and anti-wrinkling effects. Water extracts of *Arthrospira* with a high magnesium salt content were found to facilitate both ATP and matrix protein synthesis, resulting in a stimulation of keratinocyte differentiation (Schlotmann et al. 2005). The proportion of those extracts is typically in the range of 2–10% of the final formulation.

Amino acids represent 40% of the group of natural moisturizing substances and contribute to the hydration of the corneous layer cells by holding water. In cosmetic products they are being used for regulating softness, flexibility and elasticity of the skin. Our investigations have shown that hot-water extracts of *Arthrospira* contain particularly high amounts of amino acids, since during extraction the water soluble proteins are being degraded. These extracts are therefore frequently used as moisture regulating products. In recent years mainly aqueous and aqueous-ethanolic extracts have been applied in different cosmetic products such as creams, lotions, sun and hair care. For lipid-based cosmetics, like creams or lotions, supercritical CO_2-extracts are gaining commercial importance, because toxic solvents can be avoided. Mendiola et al. (2007) described an antibacterial and antifungal CO_2 extract from *A. platensis*, and also a tocopherol- enriched extract (Mendiola et al. 2008), both of which are of potential interest for cosmetic preparations. Table 26.5 compares

marketed and potential products containing active ingredients from *Arthrospira* with those from eukaryotic algae.

Aphanizomenon and *Arthrospira* produce high molecular weight polysaccharides, which have been reported to have in vitro higher immunostimulatory activities than commercially available immunotherapeutica (Pugh et al. 2001). As an example calcium spirulan (Ca-Sp) is of particular interest for its use in cosmeceuticals. A 3-step purification process for the polysaccharide from *Arthrospira* has been developed and high anti-HSV activities detected (Sandau and Pulz 2009). Ca-Sp stimulates the metabolic activity of human scin fibroblast cell lines (NHDFc), which are responsible for collagen synthesis and firmness of the skin. With increasing age the collagen synthesis drops significantly, so a main target of cosmetic research is the development of anti-aging products capable of enhancing the metabolic activity of fibroblasts, such as shown for Ca-Sp. A 36% enhancement of collagen synthesis was found by applying Ca-Sp at 10 μg mL^{-1} (Sandau and Pulz 2009). It was also found that UV-A exposed fibroblasts showed a higher vitality, if Ca-Sp had been added prior to or even after radiation. Although the protective mechanisms have not yet been studied, the application of *Arthrospira* extracts or purified Ca-Sp seems promising for different cosmetic products, with an emphasis on anti-aging and sun screens, as well as on anti-HSV lipsticks.

Cyanobacteria are exposed to high oxygen and radical stresses, especially in extreme environments. This has resulted in the development of numerous efficient protective systems against oxidative and radical stressors (Whitton and Potts 2000). The protective mechanisms are able to prevent the accumulation of free radicals and reactive oxygen species and thus to counteract cell damaging activities. Because the antioxidative components originate from a natural source, their application in cosmetics for preserving and protecting purposes is developing rapidly. Since exposure of the skin to UV light is one of the main reasons for premature skin aging and also for skin cancer, sun-protecting cosmetics represent an area of high demand. Many cyanobacteria are capable of overcoming the toxicity of ultraviolet radiation by synthesizing UV-absorbing compounds (Chap. 19). The strongest UV-A-absorbing compounds in nature are the water-soluble mycosporine-like amino acids (MAAs) e.g. shinorine. They are small (<400 Da) molecules, consisting of cyclohexinimine or cyclohexenone, their synthesis being induced by UV-B radiation (Sinha et al. 2001). Other powerful UV-absorbing natural compounds are the scytonemins. These are lipid-soluble indole alkaloids of yellow brown colour found exclusively in cyanobacteria and accumulate in the polysaccharide sheath. The conjugated double bond system absorbs UV-A radiation so that they act as photo-protectants (Rastogi and Sinha 2009). These compounds are biotechnologically exploited by the cosmetic industry for the development of sunscreens.

In general the cosmetic market segment is changing rapidly and new products with skin- protecting characteristics are welcome to the industry. The almost untapped potential of the cyanobacteria with their vast adaptation mechanisms is of particular interest in this connection.

26.4.5 Biofertilizers

Macroalgae are used as soil fertilizer in coastal regions all over the world (Critchley and Ohno 1998; Zemke-White and Ohno 1999). The role especially of cyanobacteria in the soil ecosystem has thereby often been neglected. The main beneficial effects are numerous: increased water-binding capacity and water storage, particle adherence and decreased soil erosion, improvement of mineral composition of the soil, the production and secretion of bioactive compounds such as phytohormones, which stimulate the growth of agricultural crops (Stirk et al. 2002). These properties can also be used in liquid fertilizers produced from the macroalgae, such as in the development of cover for abandoned mining lands to avoid erosion and to initiate floral succession.

In the last years numerous studies have been carried out in order to include strain identification, isolation and culture, analyzing their N_2-fixing activity and related physiology, biochemistry, and energetic as well as the structure and regulation of nitrogenfixing (nif) genes and nitrogenase enzyme (Vaishampayan et al. 2001). Due to their wide tolerance of adverse environmental conditions like desiccation, hot temperatures etc. cyanobacteria appear to be particularly suitable for the use as fertilizers.

There have also been many studies on the use of cyanobacteria, but in this case usually as inocula to encourage the growth of particular species rather than effects due to the whole biomass. Various potentially other useful effects have been shown for the cyanobacteria, such as antifungal substances (Kim 2006). Pre-soaking rice seed with cyanobacterial cultures or extracts has been reported to enhance germination and growth, although consistent investigations are still lacking (Sharma et al. 2010). Overwhelmingly, however, it has been the ability of some cyanobacteria to fix N_2 which has the main interest. Some of the many accounts of the properties of N_2-fixing organisms and isolates from soils, especially rice-fields, were reviewed by Whitton and Potts (2000) and Vaishampayan et al. (2001). The study of N_2 fixation in rice fields of north-east Spain by Quesada et al. (1997) provides an example of the importance of cyanobacterial fixation; they estimated that N_2 fixed on a per crop basis was in the range of 5–>80 kg ha^{-1} N, the value beingly strongly influenced by environmental conditions. Vaishampayan et al. (2001) quoted an average of 20–30 kg N $crop^{-1}$ ha^{-1}. This can lead to a reduction in the need for N fertilizer. Using *Aulosira fertilissima* and *Anabaena*

doliolum with or without the combination of urea, the chemical N demand for a rice field in north India could be reduced by 25% (Dubey and Rai 1995). While cyanobacterial N_2 fixation to enhance crop yields and reduce use of N fertilizers, cyanobacteria can also be used in more arid regions to reduce erosion processes, because of their formation of EPS, which improves water-binding capacity and soil structure (Chap. 12). Some of the organisms used for this are also N_2-fixers.

Among the numerous studies on the use of cyanobacterial inocula, perhaps the most important aspect is whether or not they make use of indigenous strains. The study in Chile by Pereira et al. (2009) did incorporate *Anabaena iyenganii* and *Nostoc* spp. indigenous to the region in their biofertilizer for trials on local rice fields. The use of the fertilizer decreased the amount of synthetic nitrogen fertilizer (50 kg N ha^{-1}) required by as much as 50%, while still resulting in the same yield of 7.4 t ha^{-1} rice.

An alternative approach is to make use of *Azolla* with its symbiotic *Anabaena* (Chap. 23). This has been done for green manuring rice fields in China and Vietnam for centuries (Watanabe 1982). There are two principle methods used in various locations for manuring with *Azolla* (Sharma et al. 2010). It can be grown as a monocrop and incorporated to the paddy prior to the rice being planted; it can also be grown together with the rice. The rice yield is thereby enhanced by 0.5–0.75 t per ha. Nevertheless it has been shown that free-living cyanobacteria release ammonium into the water, where it can be utilized by the crop, whereas with *Azolla* the ammonium is not as directly available.

Although phytohormones and growth regulators have been recognized in cyanobacteria for a long time, they have gained increasing attention during the past decade (Tarakhovskay et al. 2007). More recent accounts include indole-3-acetic acid (IAA) in *Nostoc* sp. (Sergeeva et al. 2002) and gibberellin-like plant growth regulators in *Scytonema hofmanni* (Rodríguez et al. 2006); the latter reduced NaCl-induced growth inhibition in rice. IAA and cytokin were released to the growth medium by *Chroococcidiopsis* sp. Ck4 and *Anabaena* sp. Ck1 under both axenic and field conditions (Hussain and Hasnain 2011). Germination, shoot length, tillering, number of lateral roots, spike length, and grain weight were significantly enhanced in wheat. The authors concluded that cyanobacterial phytohormones are a major tool for improved growth and yield in wheat.

The results available indicate the strong potential for cyanobacterial biofertilizer technology in rice-growing countries, and applied biotechnology should contribute its part to increase biomass production so that the requirement for inorganic N can be reduced. A future trend seems to be the use of cyanobacteria against plant diseases caused by fungi, viruses or bacteria.

26.4.6 Wastewater and Exhaust Gas Treatment

The protection and preservation of the natural basis of life are not only ethical demands, but also essential for durable economic and social development. They create technological progress and jobs. Further development and improvement of existing systems for wastewater treatment, the reduction of problematic emissions and the need for water recycling are important objectives.

Micro- and macroalgae, sometimes in combination with other microorganisms, are utilized to treat wastewater and other effluents. Current uses include: removal of nutrients from circulating process water, use of CO_2 from industrial exhaust gas; disposal of contaminants from agricultural wastewater; purification of wastewater from biogas production; tertiary wastewater purification.

Governments and energy companies worldwide are showing an increasing interest in CO_2-fixation biotechnology, especially due to the introduction of CO_2 certificates. For example, Germany together with the EU, USA and Australia are conducting research efforts to find economically feasible processes for applying microalgae in environmental protection and CO_2-fixation (Pedroni et al. 2001). The cement industry, as one of the largest CO_2-emitting branches, is undertaking several investigations on how to use algae for the fixation of CO_2 (Ferey et al. 2010; Borkenstein et al. 2011).

In Germany, several projects have been completed to use both stack gas and condensed water out of this gas to produce microalgal biomass. The process has been scaled up to photobioreactor volumes of 2–6 m^3 and shown to be feasible, but not yet economic (Pulz and Gross 2004; Ferey et al. 2010). At a cement plant, both salt and freshwater algae can be cultivated using the CO_2 in the stack gas as the sole C source. Growth rates in terms of volumetric biomass productivity are high and comparable to cultures grown on pure CO_2. No harmful effects on growth or cell death could be detected within an experimental period on a cement plant in Southern France cultivating the eukaryotic algae *Nannochloropsis* sp. and *Scenedesmus* sp. (Ferey et al. 2010).

Several cyanobacteria have been used in the past decade for the treatment of agricultural or industrial wastewater. *Arthrospira* was used for pig wastewater treatment in Mexico (Olguín et al. 2003) after dilution with sea water, while *Nostoc muscorum* and *Anabaena subcylindrica* were used for industrial wastewater effluents in Egypt (El-Sheekh et al. 2005). In the latter case the growth rates were higher than in standard synthetic media. The main problem is the sterilization step prior to cyanobacterial cultivation, which hampers volumetric flow and economic feasibility. Markou and Georgakakis (2011) reviewed the utilization of cyanobacteria for the reduction of organic and inorganic load with an emphasis on *Arthrospira*. As the biogas production by

anaerobic fermentation of crops and wastes or sludges is established, cyanobacteria can be used in a second biological stage for the purification of the biogas while utilizing CO_2 for their growth and enhancing hereby the methane content in the biogas. This approach has been sucessful for *Arthrospira platensis* (Converti et al. 2009; Travieso et al. 1993) with high C utilization efficiencies. The combination of anaerobic digestion and the cultivation of microalgae seems promising and will support development of large-scale cultures (Sialve et al. 2009).

Micro- and macroalgae both have considerable ability to adsorb metals and there is considerable potential for their use in treating wastewater polluted by heavy metals. In general non-viable cells are able to adsorb more heavy metal ions than living cells, while the sorption capacity can be enhanced by pretreatment of the biomass with $CaCl_2$ (Mehta and Gaur 2005) or NaOH (Nagase et al. 2005). There are two mechanisms as basic steps responsible for the heavy metal removal effect: passive adsorption to the cell surface and the active uptake into the cytoplasm. For the adsorption process negatively charged groups (e.g. carboxyl-COOH) of the cell wall as well as functional groups of extracellular polysaccharides are available (De Philippis et al. 2003), a fast process where equilibrium is reached within minutes. The biosorption inside of the cell happens by binding of the cations to ligands, phytochelatins and metallothioneins, taking several hours before maximum uptake rate is reached. Whereas the adsorption processes are dependent upon temperature and pH, biosorption is influenced by the sum of the environmental factors that impair the metabolism of the cell. Compared with other biosorbents such as bacteria and fungi, algae show high sorption capacities and efficiencies to remove heavy metal cations as well as favourable sorption kinetics (Langmuir or Freundlich adsorption isotherms), indicating a chemosorption rather than physical adsorption (Chojnacka et al. 2005). The advantage of cyanobacteria in the field of biosorption of heavy metals is the fact that the biomass is locally and available cheaply in developing countries like China. Throughout the literature *Arthrospira* has been reported to be a very efficient biosorbent for lead, cadmium, chromium and copper cations. Gong et al. (2005) investigated the lead biosorption capacitiy of *A. maxima*; removal rates of 92% were obtained at pH 5.5. Investigation on Cr (VI) loaded wastewaters with *Lyngbya putealis* by Kiran and Kaushik (2008) and *Nostoc muscorum* by Gupta and Rastogi (2008) showed high removal ability and regeneration efficiency. *Oscillatoria laetevirens* and *Oscillatoria trichoides* were studied for their Cr^{6+} removal efficiency and comparably high sorptive capacities of 21.88 mg g^{-1} and 38.7 mg g^{-1} have been found. Living biomass showed a higher sorption capacity as dead biomass, tolerating Cr^{6+} concentrations of 30 mg L^{-1} (Miranda et al. 2012). Cd-selective adsorption ability was investigated in *Tolypothrix tenuis*, *Anabaena variabilis* and *Microcystis aeruginosa* and alkaline treatment was found to be a useful technique for producing biosorbents with highly specific binding abilities for heavy metals (Nagase et al. 2005). *Lyngbya taylorii* exhibited high uptake capacities of 1.47 mmol Pb, 0.37 mmol Cd, 0.65 mmol Ni and 0.49 mmol Zn per g dry biomass (Klimmek et al. 2001). Phosphorylation of the biomass enhanced uptake of metal ions by a factor of 2.1–6.8 by enhancing the anion density of the raw material. This additional step led to 637 mg Pb g^{-1} dry biomass, the highest value for any of the metals.

The EPS produced by many cyanobacteria are of special interest for wastewater treatment. Those anionic heteropolymers have been shown to remove heavy metals very effectively. In *Cyanothece* CE 4 and *Cyanospira capsulata* a very fast recovery of Cu^{2+} was observed, based on the anion density (De Philippis et al. 2001). The high removal capacity probably relates to the high proportion of uronic acid monomers in the polysaccharide fraction that is binding cations to its carboxyl moieties and which can reach 80% in *Cyanothece* (De Philippis and Vincenzini 1998).

Research dealing with heavy metal decontamination of aqueous or organic-aqueous solutions by hybrid biofilters, consisting of an inorganic oxide matrix and an algae biomass led to a patent (Böttcher et al. 2006) and by polyurethane foam, containing inactivated algae biomass or extraction residues to another (Falke et al. 1999). For the hybrid biofilter the combination of the two materials enhanced removal time so that shorter residence times could be realized. Good removal rates for lead and uranium have been achieved by the use of different micro- and macroalgae biomasses e.g. *Arthrospira*, *Scenedesmus*, *Fucus*, *Chlorella* and *Porphyridium*. Zhang et al. (2012) investigated the biodegradation of γ-hexachlorocyclohexane (lindane), a persistent toxic pesticide by *Anabaena azotica* isolated from Chinese paddy soil. Concentrations of 0.2 mg L^{-1} were well tolerated by the cultures and a removal of 48.8% was measured after 5 days of cultivation when grown on nitrate. The capability of *Anabaena azotica* to degrade, most likely by dechloriniation, opens the potential use in the bioremediation of contaminated soils.

The application of algal biosorbents is applicable if conventional processes of heavy metal disposal at relatively low heavy metal concentrations are uneconomic. The use of immobilized microalgae in these processes offers significant advantages with respect to solid liquid separation and accelerated reaction rates (Mallick 2002; Mehta and Gaur 2005) and is therefore applicable, as shown for *Arthrospira* by Patnaik et al. (2001). Especially in countries, where ternary wastewater treatment facilities have not been fully established yet, and cyanobacterial biomass is available cheaply, this application shows great potential and should be developed further in the near future. Additionally, they may

be added to conventional treatment facilities as "safety filters". Mobile plants for the treatment of contaminated surface, ground and wash waters would also be feasible.

26.5 Future Potential

26.5.1 Perspective

The increasing world population challenges our handling of available resources and the techniques for the production of food, feed and energy. There is little future potential for cyanobacterial biotechnology, unless the following are achieved: (1) an increase of annual biomass output in the range of at least 100-fold; (2) lowering the production costs; (3) development of new products. Valuable parts of algal biomass need to be incorporated in new, functional food and feed products in order to create markets that do not yet exist. There will be a need to adopt multidisciplinary approaches for high yielding biorefinery steps and a multi-product production strategy for cyanobacterial biomass.

The production of *Arthrospira* started with the claim to provide a cheap source of nutrition in order to feed the hungry. However, both cost and availability counteract this initial idea. The price of *Arthrospira* biomass is at least 14 times higher than for conventional plant based nutrition sources (Fig. 26.10) and 3 times higher than fish meal. Moreover the acceptance of the biomass by people that do not regularly consume algal biomass is very poor. It should be noted, however, that production of this biomass is not competing with land for food supply, which soon will become a major political question.

Nevertheless, the biotechnology of cyanobacteria is gaining momentum, especially in the preparation of valuable substances and in the field of bioenergy. Cyanobacteria have some potential for the production of biopolymers since some accumulate large amounts of poly-β-hydroxybutyrate (PHB) under certain cultivation conditions (e.g. *Nostoc muscorum* up to 21.5% of dry weight) (Haase et al. 2012). A quite new approach is the use of cyanobacteria for the intracellular bioconversion of metal ions to nanorods that are applicable in the in the development of biosensors and bio-imaging tools and have even potential as therapeutic agents (Parial et al. 2012). The nanoparticle synthesis of auric ions (Au3+) to gold nanorods was recently demonstrated in growing filaments of *Nostoc ellipsosporum*. The biotechnological production of renewable raw materials as an alternative to fossil resources or new bioconversion strategies will contribute to the enhanced cultivation of cyanobacterial biomass in the future. Both, the literature and patent situation imply optimistic developments for the future, some of which are highlighted in this section.

26.5.2 Bioactive Metabolites

Cyanobacteria are widely distributed in habitats ranging from aquatic to terrestrial environments as well as extreme habitats such as hot springs, hypersaline waters, deserts, and polar regions (Whitton and Potts 2000). As one adaptation strategy they produce a wide variety of chemically unique secondary metabolites with biological activities that include antiviral, antibacterial, antifungal, antimalarial, antitumoral or anti-inflammatory properties. Chemically, these compounds represent a wide range of drugs, including peptides, alkaloids and indole alkaloids, polyketides and terpenes (Table 26.6). The cyanobacteria have proved to be one, if not the, richest source of such bioactive metabolites (Sivonen and Börner 2008). Sharma et al. (2010) summarize

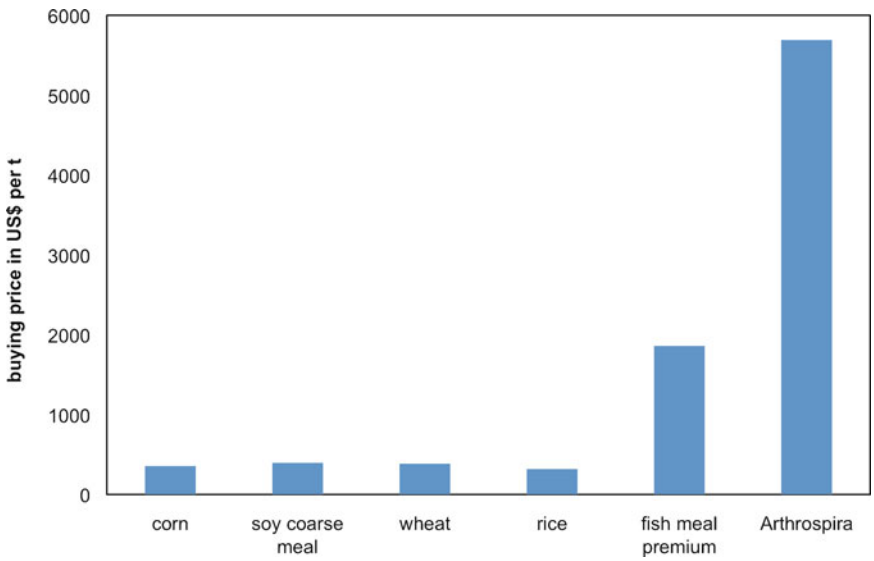

Fig. 26.10 Current buying prices for feeding stuff commodities compared to *Arthrospira platensis* biomass (W, Lehmann, 11.5.2011; Spezialfuttermittelwerk Beeskow, Germany; personal communication)

Table 26.6 Bioactive secondary metabolites isolated from cyanobacteria

Substance class	Chemical class	Examples	Biological activity	Mode of action	Organism	References
Depsipetide/polyketide	Cryptophycin	Cryptophycin 1	Anticancer	Inhibition of tubulin polymerization	*Nostoc* spp.	Trimurtulu et al. (1994)
		Cryptophycin 24	Antifungal			
		Cryptophycin 54				
Depsipeptide	Cyanopeptolins	Cyanopeptolin 1067A	Cytotoxic	Protease inhibitors	*Scytonema hofmanni* PCC 7110	Grewe (2005) and Gademann and Portmann (2008)
Depsipeptide	Microviridin	Microviridin J	Toxic to *Daphnia*	Protease inhibitors	*Microcystis viridis*	Rohrlack et al. (2003)
Depsipeptide		Largazole	Cytotoxic antiproliferative	HDAC inhibitor	*Symploca* sp.	Taori et al. (2008)
Depsipeptide	Lyngbyastatins	Lyngbyastatin 1	Cytotoxic	Serin protease inhibitor	*Lyngbya* spp.	Harrigan et al. (1998a)
			Anti-inflammatory			
			Anti-arthritic			
			Anticancer			
Cyclic peptide	Cyanobactins	Lyngbyatoxin	Tumor promotion inflammatory	Protein kinase C activation	*Lyngbya majuscula*	Jones et al. (2009)
Polyketide-peptide	Jamaicamides	Jamaicamide A	Cytotoxic	Sodium channel blocking	*Lyngbya majuscula*	Edwards et al. (2004)
Peptide	Microcystins		Antifungal	Inhibition of protein phosphatase	*Anabaena* spp.	Gupta et al. (2012)
Peptide	Symplostatins	Symplostatin 1	Anticancer	Antimitotic, inhibits cell proliferation	*Symploca hydnoides*	Harrigan et al. (1998b)
Peptide	Symplostatins	Homodolastatin 16	Anticancer	Antimitotic	*Lyngbya majuscula*	Davies-Coleman et al. (2003)
Peptide	Symplostatins	Dolastatin 10	Anticancer	Antimitotic	*Symploca* sp. VP642	Luesch et al. (2001)
Lipopeptide	Dragonamide	Dragonamide C, D	Anticancer		*Lyngbya polychroa*	Gunasekera et al. (2008)
		Dragonamide E	Antileishmanial		*Lyngbya majuscula*	Balunas et al. (2009)
Lipopeptide		Dragomabin	Antimalarial		*Lyngbya majuscula*	McPhail et al. (2007)
Lipopeptide		Spiroidesin	Anti-cyanobacterial		*Anabaena spiroides*	Kaya et al. (2002)
Thiazoline containing lipopeptide	Curacins	Curacin A	Anticancer (antiproliferative/ antimitotic)	Inhibitor of tubulin polymerization	*Lyngbya majuscula*	Gerwick et al. (1994)
Protein	Cyanovirins	Cyanovirin N	Antiviral (HIV-1, HIV-2, HSV-6, measle)	Inhibit fusion to host cells	*Nostoc ellipsosporum*	Boyd et al. (1997)
Polyketide		Borophycin	Antibiotic, anticancer		*Nostoc linckia*	Hemscheidt et al. (1994)
					Nostoc spongiaeforme	
Indole alkaloids		Hapalindole, Welwitindolinone Ambiguine	Anti-algal, antifungal and insecticidal		*Fischerella musciola, Hapalosiphon fontinalis*,	Gademann and Portmann (2008)
					H. weltwitschi	
Indole alkaloids	Bauerines, b-carboline	Bauerine A-C	Anti HIV 2		*Dichothrix baueriana* GO-25-5	Larsen et al. (1994)
Indole alkaloids	b-carboline	Norharmane	Anticyanobacterial		*Nodularia harveyana*	Volk (2005)

b-carboline alkaloid	b-carboline	Nostocarboline	Antiplasmodial	Cholinesterase inhibition	*Nostoc* 78-12A	Becher et al. (2005)
Indole alkaloide		Nostodione	Antifungal cytotoxic	Antimitotic	*Nostoc commune*	Kobayashi et al. (1994)
Indolocarbazole alkaloids	Tjipanazole	Tjipanazol A1	Antifungal		*Tolypothrix tjipanensis*	Bonjouklian et al. (1991) and Falch et al. (1995)
		Tjipanazol D	Antibacterial		*Fischerella ambigua*	
Alkaloids, Pyrrolidin-Diine	Fischerillins	Fischerellin A	Allelopathic herbicidal, antialgal antifungal	Photosystem II inhibition	*Fischerella muscicola*	Hagmann and Jüttner (1996)
Alkaloids, Indolophenanthridine	Calothrixins	Calothrixin A	Antiplasmodial anticancer		Calothrix sp.	
					Fischerella sp.	
Macrolide/lacton	Scytophycins	Tolytoxin	Anticancer, cytostatic, antifungal	Inhibition of actin polymerization	*Scytonema* sp.	Patterson and Carmeli (1992) and Patterson et al. (1993)
Sulfolipids	Sulfoquinovosyldi-acyglycerol		Anti HIV	Inhibition of reverse transcriptase	*Lyngbya lagerheimii* *Phormidium tenue*	Gustafson et al. (1989)
			Anticancer			
Porphyrin		Tolyporphin	Anticancer reversing multi drug resistance	Photosensitising	*Tolypothrix nodosa*	Prinsep et al. (1992)
Sulphated polysaccharide		Calcium Spirulan	Antiviral (HSV, cytomegaloviruses, measles, mumps, Influenza A, HIV-1) anticancer	Inhibition of penetration to host cells, inhibition of membrane invasion by tumor cells	*Spirulina* sp.	Hayashi et al. (1996) and Lee et al. (2001)
HDAC Histone Deacetylase Inhibitor	Pyrazolotriacine	Nostocine A	Antialgal cytotoxic		*Nostoc spongiaeforme*	Hirata et al. (2003)

24 novel bioactive compounds isolated from genera such as *Symploca*, *Lyngbya*, *Nostoc*, *Oscillatoria*, *Anabaena*, *Microcystis* and *Nodularia* during the decade up to the time of their review. The structurally varying metabolites are derived from mixed biosynthetic pathways and are active in the concentration range of pico- to nano-molar. More than 300 N-containing bioactive metabolites in cyanobacteria have been isolated and reported on, with the largest number being from *Nostoc* and *Symploca* (Tan 2007) details of more than 120 cyanobacterial alkaloids were published between 2001 and 2006. This research led to the identification of curacin A and dolastatin 10, which are being evaluated as anti-cancer agents or have triggered the creation of analogues (Harvey 2008). It can be summarized that cyanobacterial metabolites target specific enzymes or macromolecules related to processes that are malfunctioning in illnesses that involve cell proliferation, such as in the case of cancer. Those can be targets for the development of pharmaceuticals.

The great increase in publications in this field shows the large potential and the capability of cyanobacteria to synthesize complex metabolites with useful properties. The potential of many other compounds for clinical applications is currently under investigation. Although many of the substances isolated have potential therapeutic uses, none have yet reached clinical use. Several reasons are responsible for this. *In vivo* the activity is mostly lower or absent and any synergistic effects in raw extracts are no longer shown. At the same time toxicity may be higher. Nevertheless it seems very promising to screen and culture cyanobacteria that have not been investigated so far. New species will no doubt contribute to the finding of new substances. Neither the numerous culture collections nor more than a small number of likely habitats have been examined in depth, there is an untapped potential that awaits exploration. Cyanobacteria can serve as a prime source both for novel bioactive compounds and for leads of drugs or analogues with improved characteristics (lower toxicity, higher solubility).

A new approach is metagenomics, where synthetic abilities of organisms can be evaluated by cloning their DNA into host organisms like *E. coli*; the resulting recombinant bacteria are cultured and tested for the expression of bioactive metabolites. Molecular screening techniques can then be applied in order to identify the presence of secondary metabolite genes in species that either were not investigated or had not show bioactivity so far. Investigations on the distribution of peptide synthetase genes and polyketide synthase genes have been carried out in Nostocales, Chroococcales and Oscillatoriales. Gupta et al. (2012) investigated 28 *Anabaena* strains and identified the toxic ones by amplification of a microcystin synthase gene, linking them to previously assayed antifungal activity (see Table 26.6). Cyanobacteria can also be used for the production of recombinant proteins, such as for the treatment of diseases like HIV or to combat mosquito larvae (Sharma et al. 2010), and for the production of recombinant compounds of medicinal and commercial value. The advances in culture, screening and genetic engineering techniques have opened new ways to exploit the potential of cyanobacteria.

26.5.3 Bioenergy

The rising energy demand and the limitation of traditional sources of energy, unsettled safety issues and concerns about ecological impacts are all encouraging the development of renewable, environmental friendly energy sources. However, most studies so far have concentrated on the production of biofuel or biodiesel, for which cyanobacteria appear less suitable than eukaryotic algae, since their lipid content is much lower (on average 8%) compared to, for instance, green algae, with an average of 16–25% (Griffiths and Harrison 2009; Sialve et al. 2009). For biofuel production a high lipid productivity has been identified as a key characteristic. Nevertheless, cyanobacteria show higher solar energy conversion factors than crop plants and their easy cultivation methods, the absence of non-usable parts and comparably easy genetic manipulation protocols justify their being considered as an option for bioenergy production. The genetic transformation of *Synechococcus* PCC 6803 and *Synechococcus* PCC 7942 with pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*ad*h) genes from the bacterium *Zymomonas mobilis* for ethanol production was successfully carried out by Deng and Coleman (1999) and Dexter and Fu (2009). Fermentation of agricultural crops and residues, the common sources of bioethanol today, could be replaced by direct production of ethanol from cyanobacteria on land that is not competing with agricultural production. However, in general ethanol has some major drawbacks: a low energy density, volatility, difficulties in piping owing to its corrosive properties, hygroscopic properties necessitating energy consumptive distillation steps. Moreover the restrictive genetic engineering regulations by many state authorities will hamper scaling up this process.

Hydrogen is a possible alternative energy source that can be derived from cyanobacteria via two main biochemical pathways: mediated by bidirectional hydrogenase and by nitrogenase. A third way would be the transformation with an efficient hydrogenase from non-cyanobacteria (Angermayr et al. 2009). Hydrogen is produced within at least 14 cyanobacteria genera and under a vast range of culture conditions (Lopes Pinto et al. 2002). However, the hydrogen yields achieved today are fairly low. Problems result from hydrogen-consuming methanogens and acetogenic bacteria associated with the cyanobacteria, limited duration of production and difficulty in collecting the gas; all this is troubling its commercialization (Sharma et al. 2010).

The production of biofuel or hydrogen from algae in general, including cyanobacteria is not yet commercially feasible. The high investment costs for plants large enough to produce reasonable amounts of lipids and the subsequent high production and downstreaming costs represent the largest drawbacks. Based on the existing knowledge and technology, much future improvement and progress needs to be done before bioenergy from cyanobacteria will become a commercial commodity. The need for cost reduction in the production processes is essential. Even open pond cultivation is not yet economically feasible for commodity markets like fuel or human nutrition. Major drawbacks besides the high production cost are the high costs for downstreaming.

26.6 Conclusion

Although many ideas have been considered and much research work carried out, cyanobacterial biotechnology must be considered to be still in its infancy. Nevertheless, application of biotechnological processes to cyanobacteria is already established industrially, so academic and applied scientists should both be aware of their responsibility to make better use of their knowledge to promote this resource. We have only just started to tap the enormous biological resource of cyanobacterial species growing in all ecological niches.

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References

Abalde J, Betancourt L, Torres E, Cid A, Barwell C (1998) Purification and characterization of phycocyanin from the marine cyanobacterium *Synechococcus* sp. IO9201. Plant Sci 136(1):109–120

Abdulqader GL, Barsanti L, Tredici MR (2000) Harvest of *Arthrospira platensis* from Lake Kossorom (Chad) and its household usage among the Kanembu. J Appl Phycol 12(3):493–498

Akao Y, Ebihara T, Masuda H, Saeki Y, Akazawa T, Hazeki K, Hazeki O, Matsumoto M, Seya T (2009) Enhancement of antitumor natural killer cell activation by orally administered *Spirulina* extract in mice. Cancer Sci 100(8):1494–1501

Angermayr SA, Hellingwerf KJ, Lindblad P, Teixeira de Mattos MJ (2009) Energy biotechnology with cyanobacteria. Curr Opin Biotechnol 20(3):257–263

Anwer R, Khursheed S, Fatma T (2011) Detection of immunoactive insulin in *Spirulina*. J Appl Phycol. doi:10.1007/s10811-011-9757-1:1-9

Balunas MJ, Linington RG, Tidgewell K, Fenner AM, Urena LD, Togna GD, Kyle DE, Gerwick WH (2009) Dragonamide E, a modified linear lipopeptide from *Lyngbya majuscula* with antileishmanial activity. J Nat Prod 73(1):60–66

Becher PG, Beuchat J, Gademann K, Jüttner F (2005) Nostocarboline: isolation and synthesis of a new cholinesterase inhibitor from *Nostoc* 78-12A. J Nat Prod 68(12):1793–1795

Belay A (1997) Mass culture of *Spirulina* outdoors: the Earthrise Farms experience. In: Vonshak A (ed) *Spirulina platensis (Arthrospira)*: physiology, cell-biology and biotechnology. Taylor & Francis, London, pp 131–142, 233 pp

Belay A (2002) The potential application of *Spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement in health management. J Am Nutr Assoc 5(2):27–48

Belay A (2007) Production and quality assurance. *Spirulina* in human nutrition and health. In: Gershwin ME, Belay A (eds) *Spirulina* in human nutrition and health. CRC Press, Boca Raton, pp 1–26, 312 pp

Belay A, Kato T, Ota Y (1996) *Spirulina* (*Arthrospira*): potential application as an animal feed supplement. J Appl Phycol 8(4): 303–311

Benedetti S, Benvenuti F, Pagliarani S, Francogli S, Scoglio S, Canestrari F (2004) Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*. Life Sci 75(19):2353–2362

Benedetti S, Rinalducci S, Benvenuti F, Francogli S, Pagliarani S, Giorgi L, Micheloni M, D'Amici GM, Zolla L, Canestrari F (2006) Purification and characterization of phycocyanin from the blue-green alga *Aphanizomenon flos-aquae*. J Chromatogr B 833(1):12–18

Benemann JR (1992) Microalgae aquaculture feeds. J Appl Phycol 4(3):233–245

Bermejo Román R, Alvarez-Pez J, Acién Fernández F, Molina Grima E (2002) Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*. J Biotechnol 93(1):73–85

Bhaskar SU, Gopalaswamy G, Raghu R (2005) A simple method for efficient extraction and purification of C-phycocyanin from *Spirulina platensis* Geitler. Indian J Exp Biol 43(3):277

Blinkova L, Gorobets O, Baturo A (2001) Biological activity of *Spirulina platensis*. Zh Mikrobiol Epidemiol Immunobiol 2:114–118

Bonjouklian R, Smitka TA, Doolin LE, Molloy RM, Debono M, Shaffer SA (1991) Tjipanazoles, new antifungal agents from the blue-green alga *Tolypothrix tjipanasensis*. Tetrahedron 47(37): 7739–7750

Borkenstein CG, Knoblechner J, Frühwirth H, Schagerl M (2011) Cultivation of *Chlorella emersonii* with flue gas derived from a cement plant. J Appl Phycol 23(1):131–135

Böttcher H, Franke H, Kallies KH, Kiessig Pompe W, Sandau E, Soltmann U (2006) Heavy metal decontamination by hybrid biofilters. WO00/2006/081932, 10.08.2006 (Patent application)

Boussiba S, Richmond AE (1979) Isolation and characterization of phycocyanins from the blue-green alga *Spirulina platensis*. Arch Microbiol 120(2):155–159

Boussiba S, Richmond AE (1980) C-phycocyanin as a storage protein in the blue-green alga *Spirulina platensis*. Arch Microbiol 125(1):143–147

Boyd MR, Gustafson KR, McMahon JB, Shoemaker RH, O'Keefe BR, Mori T, Gulakowski RJ, Wu L, Rivera MI, Laurencot CM (1997) Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrob Agents Chemother 41(7):1521

But PP-H, Cheng L, Chan PK, Lau DT-W, But JW-H (2002) *Nostoc flagelliforme* and faked items retailed in Hong Kong. J Appl Phycol 14:143–145

Cai YA, Murphy JT, Wedemayer GJ, Glazer AN (2001) Recombinant phycobiliproteins 1: recombinant C-phycocyanins equipped with affinity Tags, oligomerization, and biospecific recognition domains. Anal Biochem 290(2):186–204

Cain KD, Grabowski L, Reilly J, Lytwyn M (2003) Immunomodulatory effects of a bacterial derived 1, 3 glucan administered to tilapia (*Oreochromis nilotocus* L.) in a *Spirulina* based diet. Aquac Res 34(13):1241–1244

Capelli B, Cysewski GR (2010) Potential health benefits of *Spirulina* microalgae. Nutr Food 9(2):19–26

Carlozzi P (2003) Dilution of solar radiation through "culture" lamination in photobioreactor rows facing south–north: a way to improve the efficiency of light utilization by cyanobacteria (*Arthrospira platensis*). Biotechnol Bioeng 81(3):305–315

Carmichael WW, Drapeau C, Anderson DM (2000) Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah. var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use. J Appl Phycol 12(6):585–595

Chen F, Zhang Y (1997) High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. Enzyme Microb Technol 20(3):221–224

Chen F, Zhang Y, Guo S (1996) Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnol Lett 18(5):603–608

Chen X, Smith GD, Waring P (2003) Human cancer cell (Jurkat) killing by the cyanobacterial metabolite calothrixin A. J Appl Phycol 15(4):269–277

Chen XF, Jia SR, Yue SJ, Wang N, Li CT, Wang Y (2009) Effect of solid bed-materials on vegetative cells of *Nostoc flagelliforme*. J Appl Phycol 22(3):341–347

Chen XF, Jia SR, Yue SJ, Wang Y, Wang N (2011) Biological crust of *Nostoc flagelliforme* (cyanobacteria) on sand bed materials. J Appl Phycol 23:67–71

Cheng Z, Cai H (1988) A preliminary study on the early-stage development of three species of *Nostoc*. J Northwest Norm Univ 3:41–52

Chojnacka K, Noworyta A (2004) Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme Microb Technol 34(5):461–465

Chojnacka K, Chojnacki A, Gorecka H (2005) Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue-green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere 59(1):75–84

Chuntapa B, Powtongsook S, Menasveta P (2003) Water quality control using *Spirulina platensis* in shrimp culture tanks. Aquaculture 220(1–4):355–366

Ciferri O (1983) *Spirulina*, the edible microorganism. Microbiol Mol Biol Rev 47(4):551–578

Contreras-Martel C, Matamala A, Bruna C, Poo-Caamano G, Almonacid D, Figueroa M, Martinez-Oyanedel J, Bunster M (2007) The structure at 2 Å resolution of phycocyanin from *Gracilaria chilensis* and the energy transfer network in a PC-PC complex. Biophys Chem 125(2–3):388–396

Converti A, Oliveira R, Torres B, Lodi A, Zilli M (2009) Biogas production and valorization by means of a two-step biological process. Bioresour Technol 100(23):5771–5776

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B (2005) Diverse taxa of cyanobacteria produce -N-methylamino-L-alanine, a neurotoxic amino acid. Proc Natl Acad Sci USA 102(14):5074–5078

Critchley A, Ohno M (1998) Seaweeds resources of the world. Japan International Cooperation Agency, Yokosuka

Cui Z (1983) Culture trial of facai in soil-soaked solution. Sci Technol Lett Inner Mongol 4:10–38

Cysewski GR (2010) Commercial Production of *Spirulina*. 2nd Algae World, Brussels, Belgium, 31th May–1st June

Danxiang H, Yonghong B, Zhengyu H (2004) Industrial production of microalgal cell-mass and secondary products – species of high potential: *Nostoc*. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Oxford, pp 304–311, 566 pp

Davies-Coleman MT, Dzeha TM, Gray CA, Hess S, Pannell LK, Hendricks DT, Arendse CE (2003) Isolation ofomodolastatin 16, new cyclic depsipeptide from a Kenyan collection of *Lyngbya majuscula*. J Nat Prod 66(5):712–715

De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22(3):151–175

De Philippis R, Sili C, Paperi R, Vincenzini M (2001) Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. J Appl Phycol 13(4):293–299

De Philippis R, Paperi R, Sili C, Vincenzini M (2003) Assessment of the metal removal capability of two capsulated cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936. J Appl Phycol 15(2):155–161

Delpeuch F, Joseph A, Cavelier C (1975) Consumption as food and nutritional composition of blue-green algae among populations in the Kanem region of Chad. Ann Nutr Aliment 29:497–516

Deng R, Chow TJ (2010) Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae *Spirulina*. Cardiovasc Ther 28(4): e33–e45

Deng MD, Coleman JR (1999) Ethanol synthesis by genetic engineering in cyanobacteria. Appl Environ Microbiol 65(2):523–528

DeRuyter YS, Fromme P (2008) Molecular structure of the photosynthetic apparatus. In: Herrero A, Flores E (eds) The cyanobacteria: molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 217–270, 484 pp

Dexter J, Fu P (2009) Metabolic engineering of cyanobacteria for ethanol production. Energy Environ Sci 2(8):857–864

Diao Z (1996) Study of natural conditions and ecological physiology characteristics for growth of *Nostoc flagelliforme* in Qinhai province. Chin J Ecol 15:8–13

Dias E, Pereira P, Franca S (2002) Production of paralytic shellfish toxins by *Aphanizomenon* sp. LMECYA 31 (Cyanobacteria). J Phycol 38(4):705–712

Dobler M, Dover S, Laves K, Binder A, Zuber H (1972) Crystallization and preliminary crystal data of C-phycocyanin. J Mol Biol 71(3): 785–787

Doke JM Jr (2005) An improved and efficient method for the extraction of phycocyanin from *Spirulina* sp. Int J Food Eng 1(5):1

Dubey A, Rai A (1995) Application of algal biofertilizers (*Aulosira fertilissima* Tenuis and *Anabaena doliolum* Bhardawaja) for sustained paddy cultivation in northern India. Isr J Plant Sci 43(1):41–52

Durand-Chastel H (1980) Production and use of *Spirulina* in Mexico. In: Shelef G, Soeder CJ (eds) Algal biomass, production and use. North Holland Biomedical Press, Amsterdam, pp 51–64, 852 pp

Edwards DJ, Marquez BL, Nogle LM, McPhail K, Goeger DE, Roberts MA, Gerwick WH (2004) Structure and biosynthesis of the jamaicamides, new mixed polyketide-peptide neurotoxins from the marine cyanobacterium *Lyngbya majuscula*. Chem Biol 11(6):817–833

Ehling-Schulz M, Bilger W, Scherer S (1997) UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. J Bacteriol 179(6):1940–1945

El-Sayed AFM (1994) Evaluation of soybean meal, *Spirulina* meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. Aquaculture 127(2–3):169–176

El-Sheekh MM, El-Shouny WA, Osman MEH, El-Gammal EWE (2005) Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. Environ Toxicol Pharmacol 19(2):357–365

Eriksen NT (2008) Production of phycocyanin – a pigment with applications in biology, biotechnology, foods and medicine. Appl Microbiol Biotechnol 80(1):1–14

Falch BS, Koenig GM, Wright AD, Sticher O, Angerhofer CK, Pezzuto JM, Bachmann H (1995) Biological activities of cyanobacteria: evaluation of extracts and pure compounds. Planta Med 61:321–328

Falke P, Hendreich R, Knorr G, Güttes B, Sandau P (1999) Verfahren zur Herstellung von Polyurethan-Schaumstoffen für die Adsorption von Schwermetallionen. DE000019958702

Ferey F, Walenta G, Grewe C (2010) Microalgae culture on a Lafarge cement plant. 8th workshop on microalgal biotechnology, June 7–10, Nuthetal, Germany

Fox RD (2001) The spiral of *Spirulina*. In: Trevidi PC (ed) Algal biotechnology. Pointer Publishers, Jaipur, 398 pp

Fujishiro T, Ogawa T, Matsuoka M, Nagahama K, Takeshima Y, Hagiwara H (2004) Establishment of a pure culture of the hitherto uncultured unicellular cyanobacterium *Aphanothece sacrum*, and phylogenetic position of the organism. Appl Environ Microbiol 70(6):3338–3345

Gademann K, Portmann C (2008) Secondary metabolites from cyanobacteria: complex structures and powerful bioactivities. Curr Org Chem 12(4):326–341

Gao K (1998) Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. J Appl Phycol 10(1):37–49

Gao K, Ye C (2003) Culture of the terrestrial cyanobacterium, *Nostoc flagelliforme* (Cyanophyceae) under aquatic conditions. J Phycol 39(3):617–623

Ge B, Tang Z, Zhao F, Ren Y, Yang Y, Qin S (2005) Scale-up of fermentation and purification of recombinant allophycocyanin overexpressed in *Escherichia coli*. Process Biochem 40(10):3190–3195

Gershwin ME, Belay A (2007) *Spirulina* in human nutrition and health. CRC Press, Boca Raton, 328 pp

Gerwick WH, Proteau PJ, Nagle DG, Hamel E, Blokhin A, Slate DL (1994) Structure of Curacin A, a novel antimitotic, antiproliferative and brine shrimp toxic natural product from the marine cyanobacterium *Lyngbya majuscula*. J Org Chem 59(6):1243–1245

Gong R, Ding Y, Liu H, Chen Q, Liu Z (2005) Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. Chemosphere 58(1):125–130

Görs M, Schumann R, Hepperle D, Karsten U (2010) Quality analysis of commercial *Chlorella* products used as dietary supplement in human nutrition. J Appl Phycol 22(3):265–276

Graverholt OS, Eriksen NT (2007) Heterotrophic high-cell-density fed-batch and continuous-flow cultures of *Galdieria sulphuraria* and production of phycocyanin. Appl Microbiol Biotechnol 77(1):69–75

Grawish ME (2008) Effects of *Spirulina platensis* extract on Syrian hamster cheek pouch mucosa painted with 7, 12-dimethylbenz [a] anthracene. Oral Oncol 44(10):956–962

Grawish ME, Zaher AR, Gaafar AI, Nasif WA (2010) Long-term effect of *Spirulina platensis* extract on DMBA-induced hamster buccal pouch carcinogenesis (immunohistochemical study). Med Oncol 27(1):20–28

Grewe JC (2005) Cyanopeptoline und Scytocyclamide: zyklische Peptide aus *Scytonema hofmanni* PCC7110; Struktur und biologische Aktivität. Fakultät für Biologie. Ph D thesis, Albert-Ludwigs-Universität Freiburg im Breisgau, Freiburg

Griffiths MJ, Harrison STL (2009) Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 21(5):493–507

Grobbelaar JU (2003) Quality control and assurance: crucial for the sustainability of the applied phycology industry. J Appl Phycol 15(2):209–215

Grobbelaar JU (2009a) From laboratory to commercial production: a case study of a *Spirulina (Arthrospira)* facility in Musina, South Africa. J Appl Phycol 21(5):523–527

Grobbelaar JU (2009b) Factors governing algal growth in photobioreactors: the "open" versus "closed" debate. J Appl Phycol 21(5):489–492

Guan X, Qin S, Su Z, Zhao F, Ge B, Li F, Tang X (2007) Combinational biosynthesis of a fluorescent cyanobacterial holo-α-phycocyanin in *Escherichia coli* by using one expression vector. Appl Biochem Biotechnol 142(1):52–59

Gunasekera SP, Ross C, Paul VJ, Matthew S, Luesch H (2008) Dragonamides C and D, linear lipopeptides from the marine cyanobacterium brown *Lyngbya polychroa*. J Nat Prod 71(5):887–890

Gupta V, Natarajan C, Chaudhary V, Kumar A, Sharma E, Sharma J, Bhatnagar, AK, Prasanna R (2012) Analyses of diversity among fungicidal *Anabaena* strains. J Appl Phycol. doi:10.1007/s10811-012-9793-5:1-11

Gupta V, Rastogi A (2008) Sorption and desorption studies of chromium (VI) from nonviable cyanobacterium *Nostoc muscorum* biomass. J Hazard Mater 154(1–3):347–354

Gustafson KR, Cardellina JH, Fuller RW, Weislow OS, Kiser RF, Snader KM, Patterson GML, Boyd MR (1989) AIDS-antiviral sulfolipids from cyanobacteria (blue-green algae). J Natl Cancer Inst 81(16):1254

Haase SM, Huchzermeyer B, Rath T (2012) PHB accumulation in *Nostoc muscorum* under different carbon stress situations. J Appl Phycol 24(2):157–162

Hagmann L, Jüttner F (1996) Fischerellin A, a novel photosystem-II-inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity. Tetrahedron Lett 37(36):6539–6542

Harrigan GG, Yoshida WY, Moore RE, Nagle DG, Park PU, Biggs J, Paul VJ, Mooberry SL, Corbett TH, Valeriote FA (1998a) Isolation, structure determination, and biological activity of dolastatin 12 and lyngbyastatin 1 from *Lyngbya majuscula/Schizothrix* calcicola cyanobacterial assemblages. J Nat Prod 61(10):1221–1225

Harrigan GG, Yoshida WY, Moore RE, Nagle DG, Park PU, Biggs J, Paul VJ, Mooberry SL, Corbett TH, Valeriote FA (1998b) Isolation, structure determination, and biological activity of dolastatin 12 and lyngbyastatin 1 from *Lyngbya majuscula/Schizothrix calcicola* cyanobacterial assemblages. J Nat Prod 61(10):1221–1225

Harvey AL (2008) Natural products in drug discovery. Drug Discov Today 13(19–20):894–901

Hayashi T, Hayashi K, Maeda M, Kojima I (1996) Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. J Nat Prod 59(1):83–87

Helblin EW, Gao K, Ai H, Ma Z, Villafañe VE (2006) Differential responses of *Nostoc sphaeroides* and *Arthrospira platensis* to solar ultraviolet radiation exposure. J Appl Phycol 18(1):57–66

Hemscheidt T, Puglisi MP, Larsen LK, Patterson GML, Moore RE, Rios JL, Clardy J (1994) Structure and biosynthesis of borophycin, a new boeseken complex of boric acid from a marine strain of the blue-green alga *Nostoc linckia*. J Org Chem 59(12):3467–3471

Henrikson R (1989) Earth food *Spirulina*. Ronore Enterprises, Inc, Laguna Beach, 174 pp

Hernandez-Corona A, Nieves I, Meckes M, Chamorro G, Barron BL (2002) Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2. Antiviral Res 56(3):279–285

Herrera A, Boussiba S, Napoleone V, Hohlberg A (1989) Recovery of c-phycocyanin from the cyanobacterium *Spirulina maxima*. J Appl Phycol 1(4):325–331

Hill DR, Keenan TW, Helm RF, Potts M, Crowe LM, Crowe JH (1997) Extracellular polysaccharide of *Nostoc commune* (Cyanobacteria) inhibits fusion of membrane vesicles during desiccation. J Appl Phycol 9(3):237–248

Hirata K, Yoshitomi S, Dwi S, Iwabe O, Mahakhant A, Polchai J, Miyamoto K (2003) Bioactivities of nostocine A produced by a freshwater cyanobacterium *Nostoc spongiaeforme* TISTR 8169. J Biosci Bioeng 95(5):512–517

Hu Q (2004) Industrial production of microalgal cell mass and secondary products major industrial species: *Arthrospira (Spirulina) platensis*. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Oxford, pp 264–272, 566 pp

Hussain A, Hasnain S (2011) Phytostimulation and biofertilization in wheat by cyanobacteria. J Ind Microbiol Biotechnol 38(1): 85–92

Jensen GS, Ginsberg DI, Drapeau C (2001) Blue-green algae as an immuno-enhancer and biomodulator. J Am Nutr Assoc 3:24–30

Jiménez C, Cossío BR, Labella D, Niell FX (2003) The feasibility of industrial production of *Spirulina (Arthrospira)* in Southern Spain. Aquaculture 217(1–4):179–190

John DM, Whitton BA, Brook AJ (eds) (2002) The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae. Cambridge University Press, Cambridge, UK, 904 pp

Johnson HE, King SR, Banack SA, Webster C, Callanaupa WJ, Cox PA (2008) Cyanobacteria (*Nostoc commune*) used as a dietary item in the Peruvian highlands produce the neurotoxic amino acid BMAA. J Ethnopharmacol 118(1):159–165

Jones AC, Gu L, Sorrels CM, Sherman DH, Gerwick WH (2009) New tricks from ancient algae: natural products biosynthesis in marine cyanobacteria. Curr Opin Chem Biol 13(2):216–223

Kanekiyo K, Lee JB, Hayashi K, Takenaka H, Hayakawa Y, Endo S, Hayashi T (2005) Isolation of an antiviral polysaccharide, Nostoflan, from a terrestrial cyanobacterium, *Nostoc flagelliforme*. J Nat Prod 68(7):1037–1041

Kanekiyo K, Hayashi K, Takenaka H, Lee JB, Hayashi T (2007) Anti-herpes simplex virus target of an acidic polysaccharide, nostoflan, from the edible blue-green alga *Nostoc flagelliforme*. Biol Pharm Bull 30(8):1573–1575

Kaya K, Mahakhant A, Keovara L, Sano T, Kubo T, Takagi H (2002) Spiroidesin, a novel lipopeptide from the cyanobacterium *Anabaena spiroides* that inhibits cell growth of the cyanobacterium *Microcystis aeruginosa*. J Nat Prod 65(6):920–921

Khaing MK (2004) A study on the edible cyanobacteria (blue green algae) *Nostoc* species in Upper Myanmar. Ph D thesis, Department of Botany, Mandalay University, Myanmar

Khan Z, Bhadouria P, Bisen P (2005) Nutritional and therapeutic potential of *Spirulina*. Curr Pharm Biotechnol 6(5):373–379

Kim JD (2006) Screening of cyanobacteria (blue-green algae) from rice paddy soil for anti-fungal activity against plant pathogenic fungi. Korean J Mycol 34(3):138–142

Kiran B, Kaushik A (2008) Cyanobacterial biosorption of Cr (VI): application of two parameter and Bohart Adams models for batch and column studies. Chem Eng J 144(3):391–399

Klimmek S, Stan HJ, Wilke A, Bunke G, Buchholz R (2001) Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. Environ Sci Technol 35(21):4283–4288

Kobayashi A, Kajiyama S, Inawaka K, Kanzaki H, Kawazu K (1994) Nostodione A, a novel mitotic spindle poison from a blue green alga *Nostoc commune*. Z Naturforsch 49(7–8):464–470

Larsen LK, Moore RE, Patterson GML (1994) β-Carbolines from the blue-green alga *Dichothrix baueriana*. J Nat Prod 57(3): 419–421

Lee RE (2008) Phycology, 4th edn. Cambridge University Press, Cambridge, UK, 560 pp

Lee JB, Srisomporn P, Hayashi K, Tanaka T, Sankawa U, Hayashi T (2001) Effects of structural modification of calcium spirulan, a sulfated polysaccharide from *Spirulina platensis*, on antiviral activity. Chem Pharm Bull 49(1):108–110

Liaaen-Jensen S, Egeland ES (1999) Microalgal carotenoids. In: Cohen Z (ed) Chemicals from microalgae. Taylor & Francis Ltd., London, pp 145–172, 417 pp

Liao X, Zhang B, Wang X, Yan H, Zhang X (2011) Purification of C-phycocyanin from *Spirulina platensis* by single-step ion-exchange chromatography. Chromatographia 73:291–296

Liu XJ, Chen F (2003) Cell differentiation and colony alteration of an edible terrestrial cyanobacterium *Nostoc flagelliforme*, in liquid suspension cultures. Folia Microbiol 48(5):619–626

Liu Y, Liu K, Ai Y, Jiang H, Gao X, Qiu B (2012) Differential display analysis of cDNA fragments potentially involved in *Nostoc flagelliforme* response to osmotic stress. J Appl Phycol. doi:10.1007/s10811-012-9806-4:1-8

Løbner M, Walsted A, Larsen R, Bendtzen K, Nielsen CH (2008) Enhancement of human adaptive immune responses by administration of a high-molecular-weight polysaccharide extract from the cyanobacterium *Arthrospira platensis*. J Med Food 11(2):313–322

Lodi A, Binaghi L, De Faveri D, Carvalho JCM, Converti A, Del Borghi M (2005) Fed-batch mixotrophic cultivation of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyceae) with carbon source pulse feeding. Ann Microbiol 55(3):181

Lopes Pinto FA, Troshina O, Lindblad P (2002) A brief look at three decades of research on cyanobacterial hydrogen evolution. Int J Hydrog Energy 27(11–12):1209–1215

Lu J, Takeuchi T (2004) Spawning and egg quality of the tilapia *Oreochromis niloticus* fed solely on raw *Spirulina* throughout three generations. Aquaculture 234(1–4):625–640

Lu YM, Xiang WZ, Wen YH (2010) *Spirulina* (*Arthrospira*) industry in Inner Mongolia of China: current status and prospects. J Appl Phycol 23(2):265–269

Luesch H, Moore RE, Paul VJ, Mooberry SL, Corbett TH (2001) Isolation of dolastatin 10 from the marine cyanobacterium *Symploca* species VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. J Nat Prod 64(7): 907–910

Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 15(4): 377–390

Markou G, Georgakakis D (2011) Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review. Appl Energy 88(10):3389–3401

Marquez FJ, Sasaki K, Kakizono T, Nishio N, Nagai S (1993) Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. J Ferment Bioeng 76(5):408–410

Marquez FJ, Nishio N, Nagai NS, Sasaki K (1995) Enhancement of biomass and pigment production during growth of *Spirulina platensis* in mixotrophic culture. J Chem Technol Biotechnol 62(2): 159–164

Mazokopakis EE, Karefilakis CM, Tsartsalis AN, Milkas AN, Ganotakis ES (2008) Acute rhabdomyolysis caused by *Spirulina* (*Arthrospira platensis*). Phytomedicine 15(6–7):525–527

McPhail KL, Correa J, Linington RG, González J, Ortega-Barría E, Capson TL, Gerwick WH (2007) Antimalarial linear lipopeptides from a Panamanian strain of the marine cyanobacterium *Lyngbya majuscula*. J Nat Prod 70(6):984–988

Mehta S, Gaur J (2005) Use of algae for removing heavy metal ions from wastewater: progress and prospects. Crit Rev Biotechnol 25(3):113–152

Mendiola JA, Jaime L, Santoyo S, Reglero G, Cifuentes A, Ibanez E, Señoráns F (2007) Screening of functional compounds in supercritical fluid extracts from *Spirulina platensis*. Food Chem 102(4):1357–1367

Mendiola JA, García-Martínez D, Rupérez FJ, Martín-Álvarez PJ, Reglero G, Cifuentes A, Barbas C, Ibañez E, Señoráns FJ (2008) Enrichment of vitamin E from *Spirulina platensis* microalga by SFE. J Supercrit Fluid 43(3):484–489

Miki W, Yamaguchi K, Konosu S (1986) Carotenoid composition of *Spirulina maxima*. Bull Jpn Soc Sci Fish 52(7):1225–1227

Min HEP, Zhang Yin Jiang H, Hui W (1999) Effect of the *Spirulina* feed on the growth and body color of Crucian carp. J Fish Chin 2:162–168

Minkova K, Tchernov A, Tchorbadjieva M, Fournadjieva S, Antova R, Busheva MC (2003) Purification of C-phycocyanin from *Spirulina (Arthrospira) fusiformis*. J Biotechnol 102(1):55–59

Mishima T, Murata J, Toyoshima M, Fujii H, Nakajima M, Hayashi T, Kato T, Saiki I (1998) Inhibition of tumor invasion and metastasis by calciumspirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, *Spirulina platensis*. Clin Exp Metastasis 16(6):541–550

Miranda J, Krishnakumar G, Gonsalves R (2012) Cr^{6+} bioremediation efficiency of *Oscillatoria laete-virens* (Crouan & Crouan) Gomont and *Oscillatoria trichoides* Szafer: kinetics and equilibrium study. J Appl Phycol. doi:10.1007/s10811-012-9800-x:1-16

Moreno J, Vargas MA, Rodriguez H, Rivas J, Guerrero MG (2003) Outdoor cultivation of a nitrogen-fixing marine cyanobacterium, *Anabaena* sp. ATCC 33047. Biomol Eng 20(4–6):191–197

Mühling M, Harris N, Belay A, Whitton BA (2003) Reversal of helix orientation in the cyanobacterium *Arthrospira*. J Phycol 39(2):360–367

Mühling M, Belay A, Whitton BA (2005a) Screening *Arthrospira* (Spirulina) strains for heterotrophy. J Appl Phycol 17(2):129–135

Mühling M, Belay A, Whitton BA (2005b) Variation in fatty acid composition of *Arthrospira* (Spirulina) strains. J Appl Phycol 17(2):137–146

Muller Feuga A (2004) Microalgae for aquaculture: the current global situation future trends. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Oxford, pp 352–364, 566 pp

Muller Feuga A, Robert R, Cahu C, Robin J, Divanach P (2003) Uses of microalgae in aquaculture. In: Stottrup J, McEvoy L (eds) Live feeds in marine aquaculture. Blackwell Publishing Co, Oxford, pp 253–299, 336 pp

Nagase H, Inthorn D, Oda A, Nishimura J, Kajiwara Y, Park M, Hirata K, Miyamoto K (2005) Improvement of selective removal of heavy metals in cyanobacteria by NaOH treatment. J Biosci Bioeng 99(4):372–377

Narayan M, Manoj G, Vatchravelu K, Bhagyalakshmi N, Mahadevaswamy M (2005) Utilization of glycerol as carbon source on the growth, pigment and lipid production in *Spirulina platensis*. Int J Food Sci Nutr 56(7):521–528

Niu JF, Wang GC, Lin X, Zhou BC (2007) Large-scale recovery of C-phycocyanin from *Spirulina platensis* using expanded bed adsorption chromatography. J Chromatogr B 850(1–2):267–276

Olaizola M (2003) Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomol Eng 20(4–6):459–466

Olguín EJ, Galicia S, Mercado G, Pérez T (2003) Annual productivity of *Spirulina* (*Arthrospira*) and nutrient removal in a pig wastewater recycling process under tropical conditions. J Appl Phycol 15(2):249–257

Oliveira E, Rosa G, Moraes M, Pinto L (2008) Phycocyanin content of *Spirulina platensis* dried in spouted bed and thin layer. J Food Process Eng 31(1):34–50

Oliveira EG, Duarte JH, Moraes K, Crexi VT, Pinto LAA (2010) Optimisation of *Spirulina platensis* convective drying: evaluation of phycocyanin loss and lipid oxidation. Int J Food Sci Tech 45(8):1572–1578

Olvera Novoa M, Domínguez Cen L, Olivera Castillo L, Martínez Palacios CA (1998) Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquac Res 29(10):709–715

Padgett MP, Krogmann DW (1987) Large scale preparation of pure phycobiliproteins. Photosynth Res 11(3):225–235

Padyana AK, Bhat VB, Madyastha K, Rajashankar K, Ramakumar S (2001) Crystal structure of a light-harvesting protein C-phycocyanin from *Spirulina platensis*. Biochem Biophys Res Commun 282(4):893–898

Parages ML, Rico RM, Abdala-Díaz RT, Chabrillón M, Sotiroudis TG, Jiménez C (2012) Acidic polysaccharides of *Arthrospira* (Spirulina) *platensis* induce the synthesis of TNF-α in RAW macrophages. J Appl Phycol. doi:10.1007/s10811-012-9814-4:1-10

Parial D, Patra HK, Roychoudhury P, Dasgupta AK, Pal R (2012) Gold nanorod production by cyanobacteria—a green chemistry approach. J Appl Phycol 24:55–60

Patil G, Raghavarao K (2007) Aqueous two phase extraction for purification of C-phycocyanin. Biochem Eng J 34(2):156–164

Patil G, Chethana S, Sridevi A, Raghavarao K (2006) Method to obtain C-phycocyanin of high purity. J Chromatogr A 1127(1–2): 76–81

Patil G, Chethana S, Madhusudhan M, Raghavarao K (2008) Fractionation and purification of the phycobiliproteins from *Spirulina platensis*. Bioresour Technol 99(15):7393–7396

Patnaik S, Sarkar R, Mitra A (2001) Alginate immobilization of *Spirulina platensis* for wastewater treatment. Indian J Exp Biol 39(8):824–826

Patterson GML, Carmeli S (1992) Biological effects of tolytoxin (6-hydroxy-7-O-methyl-scytophycin b), a potent bioactive metabolite from cyanobacteria. Arch Microbiol 157(5):406–410

Patterson GML, Smith CD, Kimura LH, Britton BA, Carmeli S (1993) Action of tolytoxin on cell morphology, cytoskeletal organization, and actin polymerization. Cell Motil Cytoskel 24(1):39–48

Pedroni P, Davison J, Beckert H, Bergman P, Benemann J (2001) A proposal to establish an international network on biofixation of CO_2 and greenhouse gas abatement with microalgae. J Energy Environ Res 1(1):136–150

Pereira I, Ortega R, Barrientos L, Moya M, Reyes G, Kramm V (2009) Development of a biofertilizer based on filamentous nitrogen-fixing cyanobacteria for rice crops in Chile. J Appl Phycol 21(1):135–144

Posten C (2009) Design principles of photo bioreactors for cultivation of microalgae. Eng Life Sci 9(3):165–177

Preußel K, Stüken A, Wiedner C, Chorus I, Fastner J (2006) First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. Toxicon 47(2):156–162

Prinsep MR, Caplan FR, Moore RE, Patterson GML, Smith CD (1992) Tolyporphin, a novel multidrug resistance reversing agent from the blue-green alga *Tolypothrix nodosa*. J Am Chem Soc 114(1):385–387

Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS (2001) Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. Planta Med 67(8):737–742

Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65(6):635–648

Pulz O, Scheibenbogen K (1998) Photobioreactors: design and performance with respect to light energy input. In: Scheper T (ed) Bioprocess and algae reactor technology, apoptosis, vol 59. Springer, Berlin, pp 123–152, 259 pp

Pulz O, Storandt R, Boback A (2008) Mikroalgen in der Broileraufzucht. DGS 9:15–18

Pushparaj B, Pelosi E, Tredici MR, Pinzani E, Materassi R (1997) An integrated culture system for outdoor production of microalgae and cyanobacteria. J Appl Phycol 9(2):113–119

Qiu B, Gao K (2002) Daily production and photosynthetic characteristics of *Nostoc flagelliforme* grown under ambient and elevated CO_2 conditions. J Appl Phycol 14(2):77–83

Qiu B, Liu J, Liu Z, Liu S (2002) Distribution and ecology of the edible cyanobacterium Ge-Xian-Mi (*Nostoc*) in rice fields of Hefeng County in China. J Appl Phycol 14(5):423–429

Quesada A, Leganés F, Fernández-Valiente E (1997) Environmental factors controlling N_2 fixation in Mediterranean rice fields. Microb Ecol 34(1):39–48

Raja R, Hemaiswarya S, Kumar NA, Sridhar S, Rengasamy R (2008) A perspective on the biotechnological potential of microalgae. Crit Rev Microbiol 34(2):77–88

Ramos A, Acien FG, Fernandez-Sevilla JM, Gonzalez CV, Bermejo R (2011) Development of a process for large-scale purification of C-phycocyanin from *Synechocystis aquatilis* using expanded bed adsorption chromatography. J Chromatogr B 879(7–8):511–519

Rapala J, Sivonen K, Luukkainen R, Niemelä SI (1993) Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-*toxic Anabaena*-strains—a laboratory study. J Appl Phycol 5(6):581–591

Rasool M, Sabina EP (2009) Appraisal of immunomodulatory potential of *Spirulina fusiformis*: an in vivo and in vitro study. J Nat Med 63(2):169–175

Rastogi RP, Sinha RP (2009) Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnol Adv 27(4):521–539

Regunathan C, Wesley S (2006) Pigment deficiency correction in shrimp broodstock using *Spirulina* as a carotenoid source. Aquac Nutr 12(6):425–432

Richmond A, Vonshak A (1978) *Spirulina* culture in Israel. Arch Hydrobiol Beih Ergebn Limnol 11:274–280

Rickards R, Rothschild WJM, Willis AC, de Chazal NM, Kirk J, Kirk K, Saliba KJ, Smith GD (1999) Calothrixins A and B, novel pentacyclic metabolites from *Calothrix* cyanobacteria with potent activity against malaria parasites and human cancer cells. Tetrahedron 55(47):13513–13520

Rito Palomares M, Nuñez L, Amador D (2001) Practical application of aqueous two phase systems for the development of a prototype process for c phycocyanin recovery from *Spirulina maxima*. J Chem Technol Biotechnol 76(12):1273–1280

Rodríguez A, Stella A, Storni M, Zulpa G, Zaccaro M (2006) Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa* L. Saline Syst 2(1):7

Rohrlack T, Christoffersen K, Hansen PE, Zhang W, Czarnecki O, Henning M, Fastner J, Erhard M, Neilan BA, Kaebernick M (2003) Isolation, characterization, and quantitative analysis of microviridin J, a new *Microcystis* metabolite toxic to *Daphnia*. J Chem Ecol 29(8):1757–1770

Roney BR, Renhui L, Banack SA, Murch S, Honegger R, Cox PA (2009) Consumption of fa cai *Nostoc* soup: a potential for BMAA exposure from *Nostoc* cyanobacteria in China? Amyotroph Lateral Scler 10(S2):44–49

Rosello Sastre R, Posten C (2010) Die vielfältige Anwendung von Mikroalgen als nachwachsende Rohstoffe. The variety of microalgae applications as a renewable resource. Chem Ing Tech 82(11):1925–1939

Ross E, Puapong D, Cepeda F, Patterson P (1994) Comparison of freeze-dried and extruded *Spirulina platensis* as yolk pigmenting agents. Poult Sci 73(8):1282–1289

Sajilata M, Singhal R, Kamat M (2008a) Fractionation of lipids and purification of γ-linolenic acid (GLA) from *Spirulina platensis*. Food Chem 109(3):580–586

Sajilata M, Singhal RS, Kamat MY (2008b) Supercritical CO_2 extraction of γ-linolenic acid (GLA) from *Spirulina platensis* ARM 740 using response surface methodology. J Food Eng 84(2):321–326

Saker ML, Jungblut AD, Neilan BA, Rawn DFK, Vasconcelos VM (2005) Detection of microcystin synthetase genes in health food supplements containing the freshwater cyanobacterium *Aphanizomenon flos-aquae*. Toxicon 46(5):555–562

Sandau P (2010) Examples for successfully marketed active ingredients of algae in cosmetic products. Institue for Cereal Processing, Nuthetal

Sandau P, Pulz O (2009) Untersuchungen zu bioaktiven Wirkungen des Algenpolysaacharids Calcium-Spirulan aus *Arthrospira platensis*. OM & Ernährung 131:F40–F45

Santiago-Santos M (2004) Extraction and purification of phycocyanin from *Calothrix* sp. Process Biochem 39(12):2047–2052

Sarada R, Pillai MG, Ravishankar G (1999) Phycocyanin from *Spirulina* sp: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. Process Biochem 34(8):795–801

Schaeffer DJ, Malpas PB, Barton LL (1999) Risk assessment of microcystin in dietary *Aphanizomenon flos-aquae*. Ecotoxicol Environ Saf 44(1):73–80

Scherzinger D, Al Babili S (2008) In vitro characterization of a carotenoid cleavage dioxygenase from *Nostoc* sp. PCC 7120 reveals a novel cleavage pattern, cytosolic localization and induction by highlight. Mol Microbiol 69(1):231–244

Schlotmann K, Waldmann-laue M, Jassoy C, Kaeten M, Koehler E, Pulz O, Kurth E (2005) Extract of blue-green alga *Spirulina* and the use thereof in cosmetic skin-care and body care agents, EP Patent 1,239,813

Schreckenbach K, Thürmer C, Loest K, Träger G, Hahlweg R (2001) Der Einfluss von Mikroalgen (*Spirulina platensis*) in Trockenmischfutter auf Karpfen (*Cyprinus carpio*). Fischer Teichwirt 1:10–13

Sekar S, Chandramohan M (2008) Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. J Appl Phycol 20(2):113–136

Sergeeva E, Liaimer A, Bergman B (2002) Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. Planta 215(2):229–238

Sharma NK, Tiwari SP, Tripathi K, Rai AK (2010) Sustainability and cyanobacteria (blue-green algae): facts and challenges. J Appl Phycol 23(6):1059–1081

Shelef G, Soeder C (1980) Algal biomass, production and use. North Holland Biomedical Press, Amsterdam

Shi D, Zhou G, Fang S, Qiu Y, Zhong Z, Cui Z (1992) Studies on photosynthesis, respiration and morphology of *Nostoc flagelliforme*. Acta Bot Sin 34:507–514

Sialve B, Bernet N, Bernard O (2009) Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 27(4):409–416

Simon NS, Lynch D, Gallaher TN (2009) Phosphorus fractionation in sediment cores collected In 2005 before and after opnset of an *Aphanizomenon flos-aquae* bloom in Upper Klamath Lake, OR, USA. Water Air Soil Pollut 204(1):139–153

Singh NK, Parmar A, Madamwar D (2009) Optimization of medium components for increased production of C-phycocyanin from *Phormidium ceylanicum* and its purification by single step process. Bioresour Technol 100(4):1663–1669

Sinha RP, Klisch M, Walter Helbling E, Häder DP (2001) Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation. J Photochem Photobiol B Biol 60(2–3): 129–135

Sivonen K, Börner T (2008) Bioactive compounds produced by cyanobacteria. In: Herrero A, Flores E (eds) The cyanobacteria. Molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 159–197, 484 pp

Soeder C (1992) Mass cultures of microalgae – achievements and perspectives. 1st European workshop on microalgal biotechnology, Potsdam Rehbrücke, Germany

Soeder C, Muller H, Payer H, Schulle H (1971) Mineral nutrition of planktonic algae: some considerations, some experiments. Int Ver Theor Angew Limnol 19:39–58

Soni B, Kalavadia B, Trivedi U, Madamwar D (2006) Extraction, purification and characterization of phycocyanin from *Oscillatoria quadripunctulata* isolated from the rocky shores of Bet-Dwarka, Gujarat, India. Process Biochem 41(9):2017–2023

Soni B, Trivedi U, Madamwar D (2008) A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from *Phormidium fragile* and its characterization for antioxidant property. Bioresour Technol 99(1):188–194

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial Applications of Microalgae. J Biosci Bioeng 101(2):87–96

Stec B, Troxler RF, Teeter MM (1999) Crystal structure of C-phycocyanin from *Cyanidium caldarium* provides a new perspective on phycobilisome assembly. Biophys J 76(6):2912–2921

Stirk W, Ördög V, Van Staden J, Jäger K (2002) Cytokinin-and auxin-like activity in Cyanophyta and microalgae. J Appl Phycol 14(3):215–221

Storandt R, Franke H, Pulz O, Loest K, Ecke M, Steinberg KH (2000) Algae in animal nutrition. Tierernährung – Ressourcen und neue Aufgaben, Hannover, Germany, Landbauforschung Völkenrode

Su J, Jia S, Qiao C, Jung G (2005) Culture of *Nostoc flagelliforme* on solid medium. Korean J Environ Biol 23:135–140

Su J, Jia S, Chen X, Yu H (2008) Morphology, cell growth, and polysaccharide production of *Nostoc flagelliforme* in liquid suspension culture at different agitation rates. J Appl Phycol 20(3):213–217

Takenaka H, Yamaguchi Y, Sakaki S, Watarai K, Tanaka N, Hori M, Seki H, Tsuchida M, Yamada A, Nishimori T (1998) Safety evaluation of *Nostoc flagelliforme* (Nostocales, Cyanophyceae) as a potential food. Food Chem Toxicol 36(12):1073–1077

Tan LT (2007) Bioactive natural products from marine cyanobacteria for drug discovery. Phytochemistry 68(7):954–979

Taori K, Paul VJ, Luesch H (2008) Structure and activity of largazole, a potent antiproliferative agent from the Floridian marine cyanobacterium *Symploca* sp. J Am Chem Soc 130(6):1806–1807

Tarakhovskay E, Maslov YI, Shishova M (2007) Phytohormones in algae. Russ J Plant Physiol 54(2):163–170

Teas J, Hebert JR, Fitton JH, Zimba PV (2004) Algae – a poor man's HAART? Med Hypotheses 62(4):507–510

Thajuddin N, Subramanian G (2005) Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci India 89(1):47–57

Thein M (2011) The use of natural resource for sustainable production of Spirulina in Myanmar crater lakes. 5th International Algae Congress, Dec 1–2, Berlin

Tiburcio P, Galvez FCF, Cruz L, Gavino V (2007) Optimization of low-cost drying methods to minimize lipid peroxidation in *Spirulina platensis* grown in the Philippines. J Appl Phycol 19(6):719–726

Tomaselli L (1997) Morphology, ultrastructure and taxonomy of *Arthrospira (Spirulina) maxima* and *Arthrospira (Spirulina) platensis*. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): physiology, cell biology, and biotechnology. Taylor & Francis, London/San Francisco, pp 1–19, 233 pp

Tooley AJ, Cai YA, Glazer AN (2001) Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo- subunit in a heterologous host. Proc Natl Acad Sci USA 98(19):10560–10565

Toyomizu M, Sato K, Taroda H, Kato T, Akiba Y (2001) Effects of dietary *Spirulina* on meat colour in muscle of broiler chickens. Br Poult Sci 42(2):197–202

Travieso L, Sanchez E, Benitez F, Conde J (1993) *Arthospira* sp. intensive cultures for food and biogas purification. Biotechnol Lett 15:1091–1094

Trimurtulu G, Ohtani I, Patterson GML, Moore RE, Corbett TH, Valeriote FA, Demchik L (1994) Total structures of cryptophycins, potent antitumor depsipeptides from the blue-green alga *Nostoc* sp. strain GSV 224. J Am Chem Soc 116(11):4729–4737

Ugwu C, Aoyagi H, Uchiyama H (2008) Photobioreactors for mass cultivation of algae. Bioresour Technol 99(10):4021–4028

Vaishampayan A, Sinha R, Hader DP, Dey T, Gupta A, Bhan U, Rao A (2001) Cyanobacterial biofertilizers in rice agriculture. Bot Rev 67(4):453–516

Volk RB (2005) Screening of microalgal culture media for the presence of algicidal compounds and isolation and identification of two bioactive metabolites, excreted by the cyanobacteria *Nostoc insulare* and *Nodularia harveyana*. J Appl Phycol 17(4):339–347

Vonshak A (1997a) *Spirulina platensis (Arthrospira)*: physiology, cell-biology and biotechnology. Taylor & Francis, London/Bristol, 233 pp

Vonshak A (1997b) *Spirulina*: growth, physiology and biochemistry. In: Vonshak A (ed) *Spirulina platensis (Arthrospira)*: physiology, cell-biology and biotechnology. Taylor & Francis, London/Bristol, pp 43–65, 233 pp

Vonshak A, Tomaselli L (2000) *Arthrospira (Spirulina)*: systematics and ecophysiology. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publihsers, Dordrecht, pp 505–522, 669 pp

Wang ZP, Zhao Y (2005) Morphological reversion of *Spirulina (Arthrospira) platensis* (Cyanophyta): from linear to helical. J Phycol 41(3):622–628

Watanabe I (1982) *Azolla – Anabaena symbiosis* – its physiology and use in tropical agriculture. In: Dommergues YE (ed) Microbiology of tropical soils and plant productivity. Kluwer Academic Publishers, Dordrecht, p 169

Watanuki H, Ota K, Tassakka ACMAR, Kato T, Sakai M (2006) Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. Aquaculture 258(1–4):157–163

Whitton BA, Potts M (2000) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, 669 pp

Wilson AC, Punginelli C, Gall A, Bonetti C, Alexandre M, Routaboul JM, Kerfeld CA, Van Grondelle R, Robert B, Kennis J (2008) A photoactive carotenoid protein acting as light intensity sensor. Proc Nat Acad Sci 105(33):12075

Wood SA, Rasmussen JP, Holland PT, Campbell R, Crowe ALM (2007) First report of the cyanotoxin anatoxin A from *Aphanizomenon issatschenkoi* (Cyanobacteria). J Phycol 43(2):356–365

Yamamoto Y, Nakahara H (2005) The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: the importance of pH, water temperature, and day length. Limnology 6(1):1–6

Yan SG, Zhu LP, Su HN, Zhang XY, Chen XL, Zhou BC, Zhang YZ (2011) Single-step chromatography for simultaneous purification of C-phycocyanin and allophycocyanin with high purity and recovery from *Spirulina (Arthrospira) platensis*. J Appl Phycol 23(1):1–6

Yoshimura H, Kotake T, Aohara T, Tsumuraya Y, Ikeuchi M, Ohmori M (2012) The role of extracellular polysaccharides produced by the terrestrial cyanobacterium *Nostoc* sp. strain HK-01 in NaCl tolerance. J Appl Phycol 24:237–243

Yu H, Jia S, Dai Y (2009) Growth characteristics of the cyanobacterium *Nostoc flagelliforme* in photoautotrophic, mixotrophic and heterotrophic cultivation. J Appl Phycol 21(1):127–133

Zarrouk C (1966) Contribution à l étude d une cyanophycée: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima*. Paris, University de Paris, Ph.D.

Zemke-White W, Ohno M (1999) World seaweed utilisation: an end-of-century summary. J Appl Phycol 11(4):369–376

Zhang YM, Chen F (1999) A simple method for efficient separation and purification of c-phycocyanin and allophycocyanin from *Spirulina platensis*. Biotechnol Tech 13(9):601–603

Zhang H, Hu C, Jia X, Xu Y, Wu C, Chen L, Wang F (2012) Characteristics of γ-hexachlorocyclohexane biodegradation by a nitrogen-fixing cyanobacterium, Anabaena azotica. J Appl Phycol 24(2):221–225

Zhang YM, Chen F (1999) A simple method for efficient separation and purification of c-phycocyanin and allophycocyanin from *Spirulina platensis*. Biotechnol Tech 13(9):601–603

Zhong Z, Shi D, Wang F, Cui Z (1992) Effects of temperature, water content and light intensity on nitrogenase activity of *Nostoc flagelliforme*. Acta Bot Sin 34:219–225

Zitelli GC, Tomasello V, Pinzani E, Tredici MR (1996) Outdoor culture of *Arthrospira platensis* during autumn and winter in temperate climate. J Appl Phycol 8:293–301

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