Aquatic Biodiversity

Developments in Hydrobiology 171

Series editor
H. J. Dumont

Aquatic Biodiversity

A Celebratory Volume in Honour of Henri J. Dumont

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Henri J. Dumont

I first saw Henri Dumont when I was about 16 or 17 years old. He gave a public lecture on dragonflies. I forget at what occasion exactly, but it was on a winter evening, in a lecture room of the Antwerp Zoo, next to the larger theatre where now the International Queen Elisabeth contests for piano, violin and so on are organized. I was thrilled by the way Henri Dumont made the dragonflies come alive. I had been dabbling with this group for a few years then, as an undergraduate amateur, and I loved these animals, but the way Henri talked about them opened whole new worlds to me. It was then that I decided to go to the University of Ghent, to study biology, and to work with Henri Dumont. When I later told him this story, Henri claims that he had all but forgotten the occasion, which I believe immediately, as he was and is asked quite often to give popular talks about sciences, mostly about dragonflies, but also about biodiversity, sustainable development and so on. That is so, because Henri has, and always has had, a very wide interest. He is not a centipede, he is the archetypal millipede.

Henri Jean Dumont was born in Denderleeuw, a small city between Ghent and Brussels (Belgium), in 1942. When he obtained his diploma of Licenciate in Biology (similar to Honours' degree) in 1964 at the University of Ghent, he had already published his first papers. He obtained his Diploma of Doctor of Sciences (PhD) in Zoology, again at the University of Ghent, in November 1968 with greatest distinction. He became "Geaggregeerde van het Hoger Onderwijs" (DSc), the highest academic degree in Belgium, in December 1979. The entire scientific career of Henri Dumont unfolded at the University of Ghent. This may sound strange to Anglo-Saxon scientists, but it was, and to a degree still is, quite normal and common at Belgian Universities. He became Assistant Professor at the University of Ghent in 1968, Senior Assistant Professor in 1969, Associate Professor in 1975 and Senior Associate Professor in 1980. Since 1987, he has been Professor of Ecology in Ghent and held the position of Director of the Laboratory of Animal Ecology, Biogeography and Conservation between 1987 and 1994, when the function was abolished. Henri owes much to the University of Ghent, and Ghent owes a lot to Henri. Nevertheless, the relationship between both has at times been strained, as is often the case when a strong and independent mind is forced to follow rules imposed by administrators.

The first one and a half decades of Henri's career were spent nearly exclusively on research, especially organizing extensive expeditions, to the Middle East, Mount Everest and various places in Africa, but most of all to his beloved Sahara and Sahel. During each and every one of these trips, about 50 in total, he studied dragonflies, the *file rouge* through his entire career, but meanwhile he had also picked up research on various zooplankton groups: Cladocera, Copepoda, Rotifera and later also Anostraca. In addition, he also contributed to the taxonomy, ecology and zoogeography of a great many other invertebrate groups, such as limnomedusae, Bathynellacea, etc. etc, as well as on some vertebrate groups, such as fish, turtles and even crocodiles.

Because of his wide interest, Henri Dumont attracted many students in different fields; about twenty of them successfully completed a PhD. Some of these now have their own research groups, and some of them have managed to publish their results in the top journals *Nature* and *Science*. Henri himself has given an example to all of us, his ex-students, by publishing hundreds of papers, including several in *Nature*, and a good dozen books (see bibliography of Henri Dumont after this introduction). For fun, one of his associates summarized his track record from the Web of Science a few years ago: in total, he had than amassed more than 2000 citations (about 40–80 per year since 1975 and over 100 per year since 1995). Not at all bad.

In 1980, his life changed. Professor Vaas, then Editor-in-chief of *Hydrobiologia*, died unexpectedly and Wil Peters, at that stage with Dr W. Junk Publishers (now with Backhuys Publ.) invited Henri to become chief editor of this journal. It so happened that Henri was (once again) on an expedition in the Sahara and he only heard the news of the offer a few days later. In his inaugural foreword in *Hydrobiologia* he recalled that he was lying on the roof of his Land Rover, watching the dark North African sky exploding with stars, and was pondering whether or not he should accept this task. On the one hand, what a challenge! On the other hand, his 3-month expeditions through dry sand and rock, chasing small patches of isolated water bodies (springs, small lakes called 'gueltas', temporary pools and groundwater aquifers), would come to an end. So what should he do? Fun or duty? Duty or fun?

The rest is history. Henri became Editor-in-chief of *Hydrobiologia*, and changed it into a refereed journal, eventually with impact factor, and with international standing. From the initial 6 or so volumes a year, *Hydrobiologia* became a twenty-five volume journal, publishing between 5000 and 6000 pages annually. *Hydrobiologia* now is the main channel for publication of the proceedings of a variety of bi- or tri-annual meetings in limnology and oceanography. The proceedings of Rotifera, Copepoda and Cladocera meetings have (nearly) all been published in this journal. Other groups, such as the ostracodologists, have started to follow the example. Marine scientists have also discovered *Hydrobiologia*, and volumes from the seaweed symposia and on jellyfish have meanwhile found their way into the journal's issues. All of this came to be under the leadership of Henri Dumont. His editing skills equal his abilities as a scientist in general, and in various places on the globe people have told the tale of their amazement at how much Henri can improve a paper in less than 30 minutes. In this, I dare say, he is equalled by few, one exception being the late Bill Williams, a life-long friend of Henri. The record is there: more than 350 volumes appeared under his editorship, that is close to 7000 papers or about 100 000 printed pages that he accepted and edited for publication in the journal. As the rejection rate of manuscripts submitted to *Hydrobiologia* ranges between 40 and 50%, he had to read about twice that amount. Impressive, to say the least. It must be said, nevertheless, that it would have been even more difficult for him to keep things together, if it hadn't been for the continuous help of his wife, Simonne Wellekens, now a famous wine expert in Belgium. She assisted him in handling the continuous stream of manuscripts from the beginning, and for most of his term as Editor-in-chief, she was his editorial secretary.

Henri's scientific career continued to rise at the same time. He was a guest professor at the universities of Bujumbura (Burundi, 1983) and Algiers (Algeria, 1989–1991); organized 7 international meetings, and near-annual training courses for students from developing countries, first on zooplankton, later, in the framework of the United Nations University on all aspects of Biodiversity. His many and extensive duties for *Hydrobiologia* did not prevent him from accepting several other editorial responsibilities: he is also Editor-in-chief of the *International Journal of Odonatology* (Pantala), which he created himself in 1998, and is on the editorial board of a dozen other journals. He is editor of the book series *Developments in Hydrobiology*, in which close to 170 volumes have been published at this stage, of *Monographiae Biologicae*, in which 25 volumes were published under his editorship, and of the series *Guides to the Identification of the Microinvertebrates of the Continental Waters of the World*, of which the 20th volume appeared just few months ago.

Henri Dumont's hard work has been recognized by his peers, both at a national and at an international level. He received the Biannual Prize from the Belgian Government in 1981 for one of his books (on the Sahara and Sahel of course). Recently, he received the *Ordem Nacional do Merito Cientifico*, a prestigious award from the Brazilian Government. The present volume, no. 500 of *Hydrobiologia* and no. 171 of *Developments in Hydrobiology*, is a celebratory issue and is offered in the same spirit to Henri Dumont by some of his friends and colleagues, fellow chief editors, members of the editorial board, old students and others, at the occasion of his retirement as Editor-in-chief.

Because now, after twenty-three years, the circle is complete. Henri Dumont has decided to step back, to pass on the burden of the editorship. As of volume 501, Henri will no longer be Editor-in-chief of *Hydrobiologia*, but it will take years before the hydrobiological research community has fully realised this. After all, Henri WAS *Hydrobiologia* and vice versa. But 23 years of duty is very long. It is time again for some fun. Henri plans to go back to his starry starry nights in the Sahara. Certainly, he will find that the place has changed. Certainly, he will discover that he himself has also changed. A new challenge thus awaits him. Henri, from all of us, very sincerely: *bon voyage!*

KOEN MARTENS

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Freshwater ecology and biodiversity in the tropics: what did we learn from 30 years of onchocerciasis control and the associated biomonitoring of West African rivers?

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Abstract

To release humans from river blindness, the Onchocerciasis Control Programme in West Africa (OCP) was implemented in 1974 and ended in 2002. It has emphasized preservation of biodiversity and inclusion of long-term freshwater biomonitoring since its inception, a position that is unique among the other international development programmes. The biodiversity of the disease system of river blindness includes the black fly vector complex and the worm parasite. Several species of black fly vectors differ in their behaviour, which causes differences in the disease transmission processes. Likewise, different strains of the worm parasite have different pathogenic potentials and are differently transmitted by the same vector species. This complexity of the onchocerciasis disease system was not expected at the beginning of the control programme. It has been progressively discovered, partly as a result of the improvement of molecular biology techniques during the period of OCP. The biological basis for the control of the disease includes the diversity of invertebrate predators of aquatic stages of the vector as well as the sensitivity of these non-target predators to the diversity of insecticides used during OCP. Both the interspecific and intraspecific (i.e. instar) biodiversity, as well as the diversity of insecticides applied during OCP, produced a diversity of effects on the non-target invertebrates, as well as on the potential predation pressure on the vector from the predators among these non-target invertebrates. Finally two biological products, a microfilaricide drug (ivermectin) enabling chemotherapy of humans, and a biological larvicide (Bt H-14) that became available during OCP, contributed considerably to the success of OCP and provide more examples about the role of greater biodiversity in the more effective control of onchocerciasis. The biomonitoring approach designed to evaluate the environmental effects of OCP activities was also the first, longest, and largest scale biomonitoring programme ever implemented in the tropics. We discuss the criteria used to implement the long-term biomonitoring, as well as problems encountered in operational larviciding and how these were solved. Over the long term, biomonitoring faced various unexpected factors or events that made the interpretation of the results more difficult than thought at the beginning. Some of these factors could have been identified at the beginning of OCP but were underestimated, whereas others could not have been predicted. Additional research (beyond the monitoring itself) has resulted in a better understanding of the dynamics and structure of the freshwater fauna in treated as well as untreated rivers. Thus, OCP provided an opportunity to improve knowledge about the temporal dynamics and the zonation of riverine fish and invertebrate communities in West Africa. After almost 30 years of monitoring the effects of larviciding in West African rivers it can be concluded that the effect of insecticides on the aquatic fauna was usually low for fish, whereas results for invertebrates indicate changes of taxa composition and community structure even at the family level. However, this impact should not have affected the general functioning of the aquatic system.

Introduction

'Biodiversity', 'long-term variability', and 'conservation' became key concepts in ecology during the past decade. They are widely used and are considered to be especially important elements in elucidating the dynamics of ecosystems disturbed by human activities. However, the acquisition of long-term data in the tropics, as part of biodiversity-based programs, is still rare except for topics with extensive media coverage such as the loss of rainforests, or for charismatic animals such as large predators. In this context, the Onchocerciasis Control Programme in West Africa (OCP) is an exception. This programme was designed to release humans from river blindness (or onchocerciasis), but it also emphasized concern for biodiversity and implementation of long-term freshwater biomonitoring since it's inception in 1973, a position that is unique among the other international development programmes.

OCP ended in 2002 (WHO, 2002). The initial goals, the success, and the environmental issues concerning the program were all related to biodiversity. First, the biodiversity of the worm parasite and of its black fly vectors became an important issue during OCP. An understanding of the taxonomy and systematic of these biota was essential in to trying to combat onchocerciasis. Second, the biodiversity of the potential predators of the aquatic stages of the vector as well as the sensitivities of these non-target predators to the diversity of insecticides used played a major role during OCP. Third, two biological products, a filaricide drug (ivermectin) enabling the chemotherapy of humans, and a biological larvicide (*B.t.* H-14) that became available during OCP, contributed considerably to the success of OCP and provide more examples about the role of biodiversity in the control of Onchocerciasis. The biomonitoring approach designed to evaluate the environmental effects of OCP activities was also the first, longest, and largest scale biomonitoring programme ever conducted in the tropics. Throughout the programme, the selection of insecticides that were efficient against the vector but not too toxic for the non-target fauna was a permanent concern of OCP-biologists.

In this review article, we describe the role biodiversity has played in OCP, and how our understanding of the ecology of West African freshwaters (mainly rivers) was improved through research conducted during OCP. In addition we discuss the criteria used to implement the long-term biomonitoring as well as problems encountered in operational larviciding.

Onchocerciasis: biological traits of the disease system and implications for its control

Onchocerciasis is a dermal filariasis causing blindness and debilitating skin lesions. It occurs in 37 countries of which 30 are in Africa, six in America, and one in the Arabian Peninsula. Africa is, by far, the most affected continent both for the extent of the distribution and the severity of the clinical manifestations of the disease that is also called 'river blindness' (Davies, 1994).

Onchocerciasis is caused by a parasite, *Onchocerca volvulus* Leuckart. The adult female parasite (macrofilaria) is sexually active for 9–14 years, and mainly concentrates in nodules in the human skin and subcutaneous tissues (Schulz-Key, 1990; Davies, 1994). During its life span it produces millions of very small microfilariae (250–330 microns). In Africa, these microfilariae are carried from one person to another by black flies (Diptera) belonging to the *Simulium damnosum* complex. Only females of *Simulium damnosum* Théobald bite humans, and sometimes animals, because they need blood for the maturation of each batch of eggs laid (Philippon, 1977). Once the blood arrives in the black fly stomach, most of the microfilariae are digested, but a few of them pass through the intestinal wall and reach the abdominal cavity and the thoracic muscles where they transform; these microfilariae then become infective larvae (measuring 650 microns), which find their way into the mouth parts of the black fly and may thus be transmitted to humans during subsequent blood meals of the females. The maturation cycle of the larvae in the black fly takes about seven days at 27–30 °C (Plaisier et al., 1991). The number of infective larvae in a black fly is generally less than 10 and, in most cases, from one to three. When infected black flies bite humans, they deposit the infective larvae in the human skin. The larvae penetrate the skin, and nodules containing the adult worms appear between 7 and 12 months, and sometimes up to 3 years, later.

The objective of the *Simulium* control strategy was to reduce the black fly populations to a level where transmission of *O. volvulus* microfilariae is stopped, and to maintain that level until the macrofilariae in humans die out. According to results obtained in the OCP area, the mean lifespan of the adult worm initially estimated to 20 years is 14 years, so the vector control was reduced from 20 to 14 years (Le Berre et al., 1990; Hougard et al., 2001).

The vector control operations consisted of using insecticides to treat the breeding sites of rivers where larval stages of the *S. damnosum* complex develop. The great advantage of larviciding was that it attacked the vector at a stage where it is concentrated in a very small area (fast flowing parts of rapids in large rivers). In comparison, the adult black fly population cannot be controlled because of their dispersal and the wide variety of their resting places. The development of the aquatic stage from egg to pupae is around one week; hence, insecticides were applied weekly. In addition to this high treatment frequency, the large number of breeding sites along rivers that were difficult to access by ground was why an aerial spraying strategy has been retained since the beginning of the programme. At the maximum of OCP activities, up to 50 000 km of river stretches were treated during the rainy season (Hougard et al., 1997).

Onchocerciasis control as a model for the importance of biodiversity

When OCP started, taxonomical knowledge of species that were related to Onchocerciasis was limited to the vector and the parasite. As it turned out later, however, the improved understanding of the taxonomy and systematics of the vector, the parasite and many other biota that were involved in the control of Onchocerciasis contributed considerably to the success of OCP.

The biological disease system

Humans, and other mammals, are the host of many parasites that need one or two other hosts during their biological cycle. In the case of Onchocerciasis, humans are the final host, whereas black flies are the intermediate host. Understanding the epidemiology of such a disease requires a good knowledge of the biological diversity of both the vector and the parasite, as well as of the diversity of interactions among host, vector, and parasite. This understanding is fundamental to assess the transmission potential of the vector and the pathogenic potential of the infesting strains of the parasite (Toé et al., 1997). Such interaction illustrates how a complex biological system functions. While this aspect of biodiversity received little attention by biologists working mainly on ecological systems, it is certainly a biodiversity issue (Lévêque & Mounolou, 2001). However, the question in OCP was not to protect this biodiversity, but to manage it in a way to control the human disease. That was the main difference with the traditional biodiversity approach developed by conservationists and that used during OCP.

The black fly complex

The black fly vector was first described as a single species, *Simulium damnosum* Theobald. Further studies demonstrated that the species had biological differences in various biogeographic areas, and cytogenetic studies supported the evidence that *S. damnosum sensu lato* is not a single taxonomic entity. Instead, it represents a complex of sibling species, all very close morphologically, but with different chromosome structures and ecological characteristics (Le Berre, 1966; Quillévéré, 1979; Post, 1986). At first, species differentiation was based on the examination of the chromosomes of the larval salivary gland (Vajimé, 1989). DNA probes for the identification of members of the *Simulium damnosum* complex have also been used (Post & Flook, 1992). Since 1995, a technique based on mitochondrial encoded gene sequences has been developed that makes it possible to identify most species of the complex (Tang et al., 1995). Finally, microsatellite markers have been recently identified for *S. damnosum* s.l. within the OCP area, which confirmed a variable genetic structuration at the intra-specific level (Dumas et al., 1998).

Overall, nine species occur in the OCP area (Meredith et al., 1983; Boakye, 1993; Wilson et al., 1993). *S. damnosum* s.s., *S. sirbanum* and *S. dieguer-*

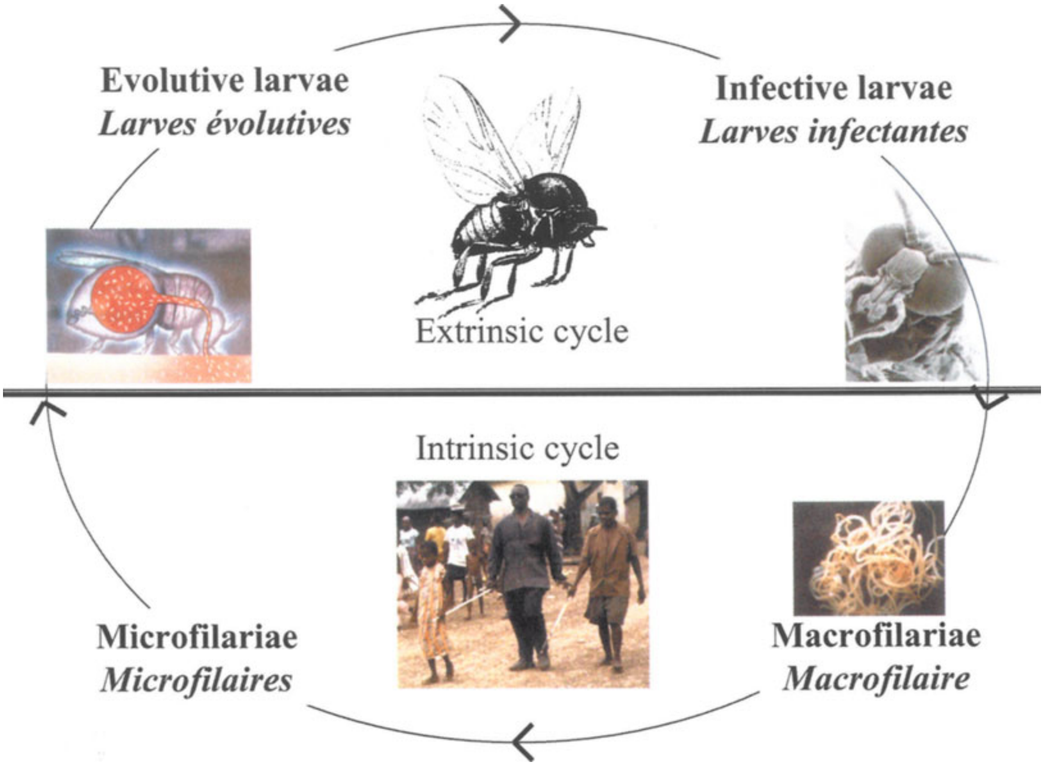

Figure 1. The life cycle of *Onchocerca volvulus*.

ense are found in the savannah zone and disappear at the northern limit of the endemic area for onchocerciasis (Fig. 2). *S. soubrense*, *S. sanctipauli*, *S. konkourense* and *S. leonense* are dense forest-dwelling species, but are also found in the zone of less dense, western forests; in some places, they even occur in savannah zones (Vajimé & Quillévéré, 1978; Garms, 1987; Garms et al., 1989). *S. yahense* is limited to small, forested watercourses, whereas *S. squamosum* is widespread in both forest and savannah zones (Quillévéré et al., 1981). These different species have different potentials for transmission of parasites; the forest vector species are more active than the savannah ones in the transmission of *O. volvulus* (Quillévéré et al., 1978).

The parasites

The complexity of the biological disease system is further increased through the existence of animal parasites as well as of several genetic strains of the human parasite. *S. damnosum* s.l. may be the host of animal microfilaria, which are morphologically very close to *O. volvulus*. In the infective stage, *O. ochengi* Bwangamoi, the main animal parasite for which *S. damnosum* s.l. is also a vector, could not be morphologically differentiated from *O. volvulus,* the human parasite. As a result, the measure of the transmission found by dissections of female black flies initially was overestimated because it took into account all the worm larval stages (Philippon, 1977). Furthermore, the clinical manifestations of the disease and the different importance of blindness it caused in forest and savannah zones suggested the existence of different strains of *O. volvulus* (Toé et al., 1997). This would have explained the differences in the pathogenic potential of the parasite observed in the forest and savannah zone. For reliable epidemiological controls, it was thus important to differentiate the different strains of the human parasite as well as the human microfilaria from those occurring in animals. DNA probes have

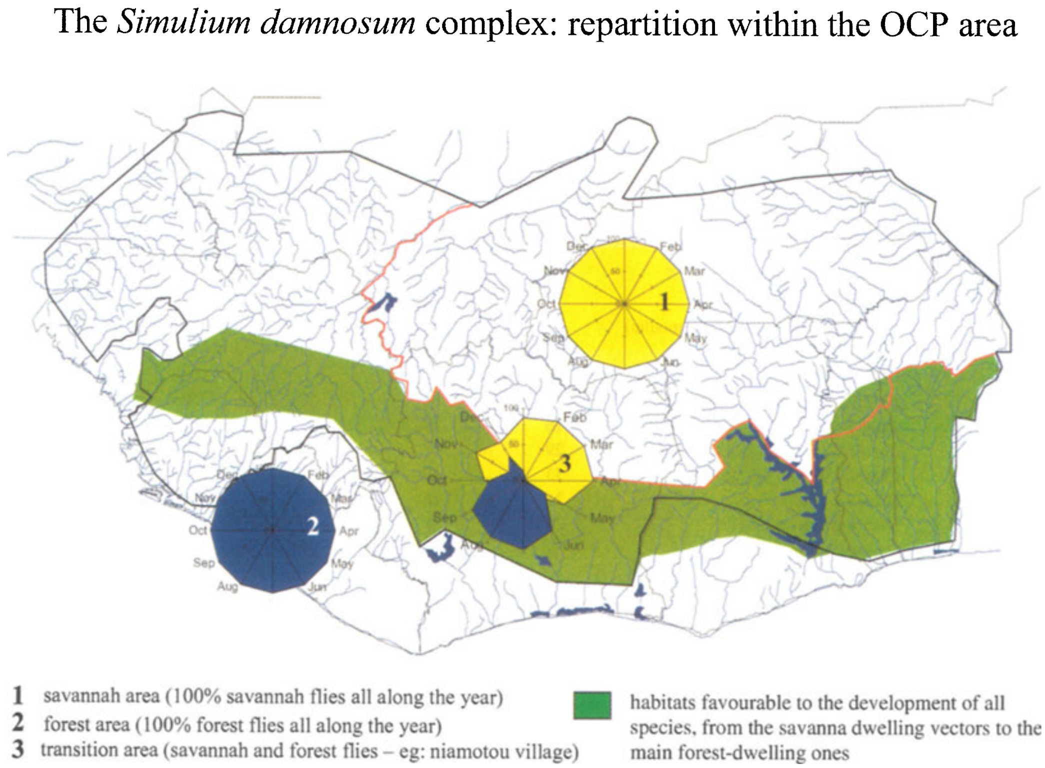

Figure 2. The *Simulium damnosum* complex: partitioning of savannah and forest species within the OCP area. (1) Savannah area (100% savannah flies all along the year: *S. damnosum*, *S. sirbanum* and *S. dieguerense* (2) Forest area (100% forest flies all along the year: *S. soubrense*, *S. sanctipauli*, *S. konkourense* and *S. leonense*. (3) Transition area where abundance of savannah and forest flies is a function of the season.

been developed (Perler & Karam, 1986; Meredith et al., 1989; Erttmann et al., 1990; Zimmerman et al., 1993; Katholi et al., 1995), and have been used by OCP since 1992. More recently, a microsatellite locus has been identified for *O. volvulus*, confirming the existence of different profiles in the allele distribution according to their geographical origin in West Africa (Dumas, 2001).

Synthesis

The above descriptions illustrate the elevated complexity caused by the biodiversity prevailing in a biological disease system over a large geographic area: (1) different vector species with different behaviour are involved, resulting in different disease transmission processes; (2) different strains of the human parasite have different pathogenic potential; and (3) human and animal parasites are transmitted by the same vector. The complexity of the biological disease system, which is similar to that of malaria or schistosomiasis for example, was not expected at the beginning of OCP. The complexity has been discovered progressively, partly as a result of the improvement of molecular techniques.

The biological 'control' system of the disease

The diversity of invertebrate predators of the larval vector and the sensibility of these predators to various insecticides

Using serological techniques, Service & Elouard (1980) identified more than 20 taxa of benthic invertebrates as predators of the black fly complex of *S. damnosum*. Among these, several species of caddisflies and a dragonfly genus (*Zygonyx*) were particular important predators of the vector (Table 1). Other taxa

Table 1. Predation of *Simulium damnosum* by various benthic invertebrates (% of animals examined containing *S. damnosum* remains in the gut or as determined by serological test) and drift loss from the benthic population as percentage of the benthic population that drifted after the insecticide application evaluated after operational and experimental treatments (at operational insecticide concentrations) with two organophosphorous insecticides (after Statzner, 1979; Service & Elouard, 1980; Elouard, 1983; Dejoux, 1988; Schorscher, 1992)

Taxon	Predation Serological tests[2]	Gut contents[3]	Drift loss[1] Temephos	Chlorphoxim
Mayflies				
Centroptiloides spp.	na[4]	31	na	na
Centroptilum spp.	4	na	24–100	na
Pseudopannota bertrandi (Demoulin)	3	na	3–100	na
Tricorythus spp.	11	na	82	73
Caddisflies				
Aethaloptera dispar Brauer	19	na	50	50
Amphipsyche spp.	16	18	71	na
Cheumatopsyche digitata (Mosely)	29	9	64	28–54
Cheumatopsyche falcifera (Ulmer)	33	10	2–76	25–48
Chimarra spp.	36	na	24–73	31–57
Macrostemum spp.	20	0	38	39
Orthotrichia spp.	0	na	35	48
Others				
Chironomidae	1	na	2–79	7–44
Elmidae	17	na	5	3
Pyralidae	0	na	1	46
Zygonyx spp.	38	25	na	na

[1] Evaluated with gutters, including benthic communities from various river reaches in the OCP-area (note that ranges are indicated if more than one value was available).

[2] As proportion of positive reactions of individuals to an antiserum of *S. damnosum*.

[3] As proportion of *Simulium* (predominantly *S. damnosum*) of all preyed invertebrate individuals.

[4] Not available.

rarely preyed on the larval vector (Table 1). Using a convential method (i.e. analysis of gut contents), Schorscher (1992) confirmed the relative importance of *Amphipsyche* spp. as a predator of the vector, but not the other two species of *Cheumatopsyche* and the species of *Macrostemum* spp. (Table 1). Specific differences in predatory behaviour in the genus *Macostemum* (e.g. *M. distinctum* [Ulmer] vs. *M. capense* [Walker]) explain the low predator impact of the species in this genus (Schorscher, 1992). The larval instars of a given species of *Cheumatopsyche*, differ considerably in their consumption of the larval vector (Table 2), i.e. the intraspecific diversity of the instar structure within a predator population at a given moment (which changes over time, Statzner, 1982; Schorscher, 1992) should determine the efficiency of predation on the larval vector.

Based on gut contents and gut clearance times of the most important predators, Schorscher (1991, 1992) estimated their maximum consumption rate at about 2500 larval *Simulium* m2 d^{-1}. Thereby, in October–December, they consumed up to 80–90% of the benthic *Simulium* population. Likewise, De Moor (1991, 1992) demonstrated that predators may effectively control black fly larvae in South Africa. As a consequence, the effect of the insecticide treatments on these predators was a particular concern during OCP.

The various chemical insecticides used by OCP differed in their chemical structure and their effects on non-target invertebrates (Yaméogo et al., 1992; Yaméogo, 1994). Two chemically closely related organophosphorous insecticides (temephos and chlorphoxim), produced similar or different effects on non-target invertebrates (including important predators of the vector [Table 1]). For example, *Aethaloptera dispar*, *Macrostemum* spp., or Elmidae had similar drift losses from the benthic population after treatments with temephos and chlorphoxim, Pyralidae had higher drift losses if treated with chlorphoxim, and

Table 2. Predation on *Simulium* (predominantly *S. damnosum*) by the larval instars of two hydropsychid (*Cheumatopsyche*) caddisfly species, expressed as mean number of larval *Simulium* consumed by 100 specimens of each instar, and drift loss from the benthic population (%) of these instars after experimental treatments with two organophosphorous insecticides (after Statzner, 1979; Elouard, 1983; Schorscher, 1992). See Table 1 for further details

Larvicide Taxon	Predation	Drift loss Temephos 0.025 ppm/ 10 min	Drift loss Temephos 0.2 ppm/ 10 min	Drift loss Chlorphoxim 0.025 ppm/ 10 min
C. digitata (Mosely)				
instar 1	0	na	na	96
instar 2	0	na	na	89
instar 3	≈ 0	na	na	26
instar 4	19	na	na	11
instar 5	26	na	na	5
C. falcifera (Ulmer)				
instar 1	0	6	29	97
instar 2	0	7	60	71
instar 3	≈ 0	6	49	21
instar 4	14	7	32	10
instar 5	20	5	8	4

some taxa (e.g. *Pseudopannota bertrandi*, *Cheumatopsyche digitata*, *C. falcifera*, Chironomomidae) had a wide range of drift losses if treated with temephos and/or chlorphoxim (Table 1). Among other reasons, biodiversity provided the key to understand these response patterns. Closely related species could differ considerably in their sensitivity to one or another insecticide (Statzner, 1979; Elouard, 1983; Wuillot, 1991), i.e. taxa aggregates above the species level should respond according to the (unknown) species included in the evaluation. In addition, different instars of a species could have similar, or rather different, responses to the treatment with a given insecticide type and concentration (Table 2) which explains that aggregates not separated at the instar level could respond according to the responses of the unknown instars included in the evaluation.

Thus, both the interspecific and intraspecific (i.e. instar) diversity, as well as the diversity of insecticides applied during OCP, produced a diversity of effects on the non-target invertebrates, as well as on the potential predation pressure on the vector black fly species.

Two biological products that contributed to Onchocerciasis control

Onchocerciasis could be controlled by chemotherapy. At the beginning of OCP, it was expected that a macrofilaricide would be available by the end of the programme to kill the adult *O. volvulus*. Unfortunately, even today, such a macrofilaricide is not still yet available. However, the microfilaricide ivermectin has proven to be effective against *O. volvulus*, and to be well tolerated, accepted, and without side effects for humans (Remme et al., 1990). Ivermectin is effective at a single dose and is, today, the only molecule used to control onchocerciasis morbidity (Abiose et al., 2000). It was originally a veterinary product and is still used as such. The human drug (trade name Mectizan) has been used for treatment in OCP since the 1980s and is the basis for Onchocerciasis elimination in the current African Programme for Onchocerciasis Control. It is another example of the role knowledge of biodiversity plays in the control of this disease in that strain of the actinomycete *Streptomyces avermitilis* was isolated from a soil sample taken from a golf course in Japan, and this resulted in the discovery of avermectins, a class of highly active antiparasitic agents from which the ivermectin drug was later derived (Lasota & Dybas, 1991).

The other biological agent, which will be discussed in detail later in this article, is *Bacillus thuringiensis* var. *israelensis H-14* (*B.t.* H-14), a biologically based larvicide that has been used in many other disease or pest control programmes. Discovered in 1977, this bacterium produces protein crystals that are toxic for *Simulium* larvae and several other Diptera. Commercial formulations were used by OCP at a large scale since 1982 (Guillet et al., 1982; Hougard & Back, 1992). The toxin of *B.t.* H-14 is indeed extremely selective for black fly larvae, and operational spraying has practically no effect on fish and relatively low effects on the non-target invertebrates. Its mode of action is unique and complex, with four polyteptides being involved that differ in mode of action that are encoded by different genes. Therefore, crossed resistances with chemical insecticides have never been recorded for *B.t.* H- 14. In fact, several rivers in the OCP area have been treated with *B.t.* H-14 for nearly 20 years without any decrease in *Simulium* susceptibility from these treatments (Hougard et al., 1997).

Synthesis

The above descriptions illustrate that biodiversity played also a major role in the biological control system. Different instars of the predator species of the vector differed in their sensitivity to the various insecticides used in OCP and consumed different quantities of *Simulium* larvae, which interfered with the predation pressure on the aquatic stages of the vector. In addition, an actinomycete and a bacterium provided the products that enabled the control of Onchocerciasis through treatments of the parasite and the vector.

The mandate of OCP for environmental monitoring

The starting point of OCP in West Africa was a meeting held in 1968 in Tunis (Le Berre et al. 1990) to discuss how this disease may be controlled, where, for how long, and how much it would cost? In reviewing the original documents arising from that 1968 meeting, it is apparent that the environment was not considered as a major issue, if an issue at all. As for centuries of applied studies, the major goal of such a disease control was to enhance the socioeconomic development. River blindness forced the rural human populations to abandon the fertile river valleys and to occupy marginally productive land away from the rivers. While these lands offered some respite from further infection, poor soils and little water hindered farming efforts. These practices led to overfarmed land and resulted in environmental degradation (Benton et al., 2002). Thus, through the control of river blindness, it was expected to have access to fertile riverside areas and to increase the agricultural productivity (Benton & Skinner, 1990).

This lack of environmental consideration at the Tunis conference was typical of the views that prevailed at that time of the 20th century (the 1950s and the 1960s) in various pest control programmes, which focused exclusively on the target organisms. In other words, the system approach was far from being a major concern to assess environmental consequences of vector control.

However, between the Tunis meeting and the beginning of OCP, environmental awareness, especially concerning the use of pesticides to control insects, increased greatly and culminated in the celebration of the first 'Earth Day' in 1970. The public and decision makers were informed through popular books such as the 'Silent Spring' from Rachel Carson (1964) about the consequences of large-scale use of DDT and other insecticides. The so-called DDT "syndrom" (Provost, 1972) had positive consequences for biodiversity: it limited the use of pesticides and stimulated impact and environmental hazard assessments. It also re-inforced the regulation about the use of pesticides. In the case of OCP, the fear of a large-scale damage in using pesticides for vector control on the long-term forced the programme to include potential environmental impacts of larviciding as a priority in operational decisions (Resh et al., in press). Actually, soon after the inception of OCP, criticism emerged around the likelihood of environmental damage (e.g. Goodland, 1974; Asibey, 1975, 1977) and concerns about environmental damage have periodically been raised throughout the life of the program (e.g. Petersen et al., 1987).

In 1974, the OCP, a 20-years onchocerciasis control campaign, was initiated at the request of seven West African countries. It was sponsored by four multilateral agencies (WHO, FAO, UNDP and World Bank) and 22 donors provided financing. Its overriding objective was to reduce the impact of onchocerciasis as an obstacle to socio-economic development (Le Berre et al., 1990; WHO, 2002). Eventually, the program area was expanded to include all or parts of 11 West African countries (Fig. 3) and also to maintain and adjust control activities to stabilize the disease at a tolerable level. It must be fully understood that the strategy was one of control, not eradication of the *Simulium* vector, as the latter an almost impossible task that was never achieved for any pest or disease vector during human history. Because of the awareness that prolonged and regular use of insect-

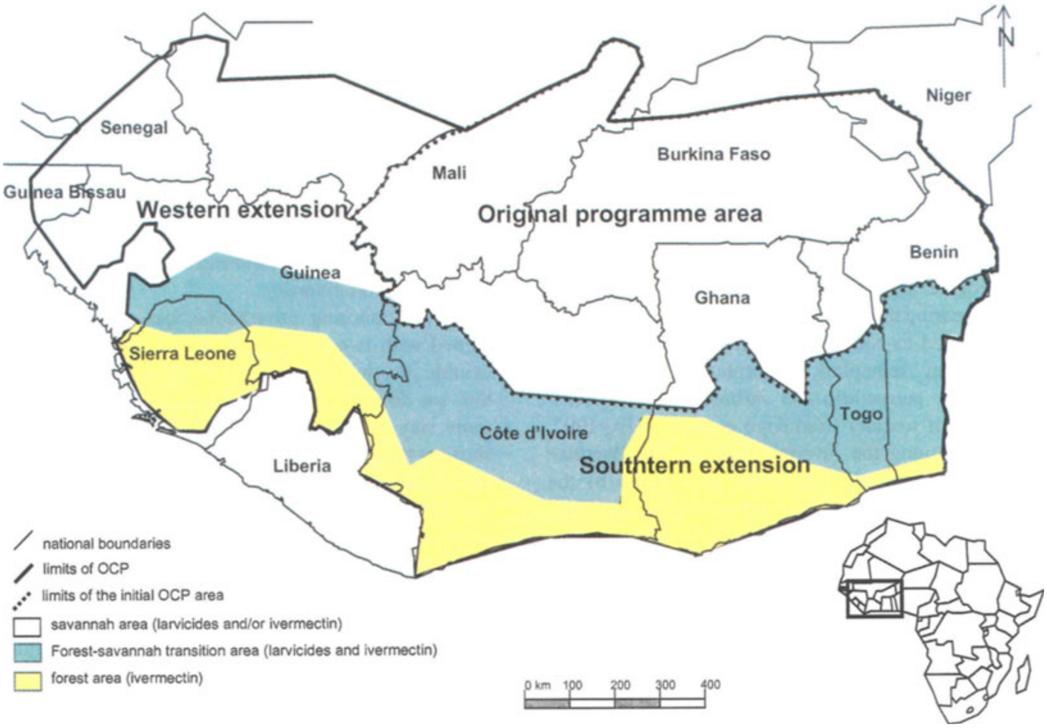

Figure 3. The Onchocerciasis Control Programme area.

icides presents a risk to the aquatic environment, an independent ecological committee, now referred to as the 'Ecological Group', was created (Calamari et al., 1998). The mandate of the Ecological Group included: (1) organization of a long-term monitoring program to evaluate changes in the aquatic fauna in relation to larviciding; (2) identification of criteria for the selection of operational insecticides, and the conditions under which they can be used in relation to seasonal and environmental factors in the different areas covered by OCP; and (3) identification of the environmental and human ecological implications of the agricultural development being undertaken in the areas previously abandoned, but now free of onchocerciasis (Calamari et al., 1998).

The short-term risk assessment of new larvicides

A risk assessment was performed for every new larvicide considered for use in OCP. After the first trials conducted to evaluate the efficacy on black flies, a literature review was performed together with laboratory tests with fish and simple gutter tests with non-target invertebrates (Calamari et al., 1998). Acute toxicological tests have been performed on African fish species according to standard protocols (Yaméogo et al., 1991a) to obtain original data on fish toxicology.

To test the immediate toxicity of insecticides on the invertebrate fauna, an original methodology was developed. At the beginning of OCP, the use of *in situ* throughs was introduced and later improved to the multi-gutters method (Troubat, 1981). These tests provided relatively accurate measures of the mortality of invertebrates after insecticide treatments and enabled comparisons of different compounds or formulations in similar, seminatural situations, because experiments could be conducted in the river itself or on its bank. Although the method provided less standardized conditions than laboratory methods, it had the advantage to be much closer to natural situations and to experiment with the autochtonous river fauna. This method was used to study the short-term impact on non-target invertebrates of different concentrations of many insecticides (Yaméogo et al., 1993). From these tests, larvicides were classified according to their general toxicity and a typology of the susceptibility of the most common non-target taxa was established (Yaméogo et al., 1991a).

Much information has also been collected from field trials of insecticides such as *B.t.* H- 14 (Dejoux, 1983; Dejoux et al., 1985), temephos (Dejoux & Elouard, 1977; Elouard & Jestin, 1983), chlorphoxim (Statzner, 1979), pyraclofos (Yaméogo, 1994), permethrin (Yaméogo et al., 1993), and etofenprox (Yaméogo et al., 2001a).

Comparing the different operational larvicides, *B.t.* H-14 proved to caused least environmental damage, followed by temephos, chlorphoxim, pyraclofos, etofenprox, permethrin and carbosulfan, in increasing order of toxicity (Yaméogo et al., 1991b, 1992, 2001b). Among the invertebrate taxa, the Baetidae (Ephemeroptera) were the most susceptible to the chemical larvicides, whereas the Chironomidae (Diptera) were the least susceptible to most of the insecticides.

The Ecological Group continuously reviewed these results. When a candidate larvicide had reasonable impact on the non target fauna, the EG recommended small-scale pilot studies in the field. For the most toxic insecticides that still had many advantages to their possible use (such as low cost, wide range of application in relation to discharges, and long 'carry' distance), large-scale studies at the operational dose were recommended. This allowed a complete risk assessment scheme to be obtained before the insecticide would be used as an operational larvicide. Chlorphoxim and permethrin, for example, were tested this way (Statzner, 1982; Yaméogo et al., 1993; Calamari et al., 1998).

The need for a long-term environmental monitoring programme

Because prolonged and intensive use of insecticides could present environmental risks, it was necessary to evaluate the possible long-term effects of such applications on the aquatic flora and fauna. In 1974, just before the beginning of operational activities, OCP set up an aquatic monitoring programme of rivers planned to be regularly treated with insecticides (Lévêque et al., 1979). It was implemented to satisfy three major concerns: (1) to provide early warning to those carrying out treatments, should toxic effects be noted at a moment, and to ensure that the insecticide release did not excessively disturb the functioning of the treated ecosystems on a long-term basis (i.e. the expected duration of OCP); (2) to avoid the widespread use of chemicals that may have adverse effects on human populations near the river systems and/or that might accumulate in the food chain, as did DDT; and (3) to prevent the irreversible loss of aquatic biodiversity in West Africa because freshwater fish are both a major source of food as well as of economic activity for West African humans. Ultimately these concerns met the objective of the Convention on Biodiversity that stipulates that countries are responsible for the conservation of their own biodiversity.

The monitoring programme was primarily concerned with two major categories of organisms: (1) benthic invertebrates that live in the watercourses and that are directly threatened by the insecticide in the same way as *S. damnosum* larvae; and (2) fish and shrimps that represent a natural resource of major interest for the people living along the river. Insecticides could affect the food chain by killing fish prey. However, repeated long-term treatments could also affect the reproduction cycle of fishes, either by direct effect on eggs and juveniles, or by indirect impact on the reproductive physiology of adults.

Special considerations had to be kept in mind when designing such a long-term monitoring programme (Lévêque et al., 1979). First, the periodicity of sampling, the sites selected for monitoring, and the field methods had to combine reliability of sampling techniques with reliability of access in both wet and dry season, over a large and, perhaps, ecologically diverse area. Second, the monitoring technique had to be standardized for use by different national teams and under various environmental conditions. Such standardisation would enable comparisons among sites. Third, when OCP was launched, the knowledge about invertebrate taxonomy as well as about the biology and ecology of animal species was limited, and the functioning of West Africa river ecosystems as a whole was poorly understood.

The criteria used by the Ecological Group for the evaluation of the long-term impact of insecticides on the aquatic environment were that (1) the vector control activities should not reduce the number of invertebrate species, or cause a marked shift in the relative abundance of species; (2) the insecticides applied should have neither a direct impact on fish, nor the life cycle of fish species; (3) bioaccumulation and biomagnification through food webs should be avoided; (4) human activities in the control area should not be impaired; and (5) temporary and seasonal variations in non-target invertebrate populations that are the results of insecticide use are acceptable.

The environmental impact of human resettlements

In terms of the environmental impact of resettlement, the success of OCP could be jeopardised by the unsustainable use of the onchocerciasis-freed land. For example, a pilot study in the Léraba area showed that 75% of the original wooded savannah was cleared for agricultural development and the settlement of villages (Baldry et al., 1995). The riverine forests of many small rivers were destroyed and, on some of the banks, soil started to erode. In other words, the resettlements in the onchocerciasis-freed areas resulted in environmental degradation from extensive forest clearing and agricultural-pesticide applications (Baldry et al., 1995). This issue of how land resettlement may threaten these onchocerciasis-freed river valleys and hamper ecological sustainability (Calamari et al., 1998) remains unsolved because the resettlement has occurred with little concern for resulting soil erosion or deforestation (McMillan et al., 1998).

Problems encountered in operational larviciding and how they were solved

The first 'ideal' larvicide: temephos

The insecticide selected for a large-scale campaign originally planned to last for about 20 years should have properties that are often contradictory, such as to have effective impact against the larvae of *S. damnosum* s.l., ease of application, low costs, few residues but a far reaching downstream effect, be harmless for humans and mammals, and the lowest possible toxicity for the rest of the aquatic environment (the non-target fauna). Temephos (Abate®), an organophosphate, more or less met the above properties, but it turned out that it could not be used during the entire OCP period.

The need to overcome the organophosphate resistance

Temephos was the only larvicide used in OCP from 1974 to 1979. After 6 years of larviciding, temephos resistance developed in some cytospecies of the *S. damnosum* complex in breeding sites of the Lower Bandama River, from where it spread rapidly to the southern forest zone and to parts of the humid savanna zone (Guillet et al., 1980; Kurtak, 1986). The resistant species in the Lower Bandama was identified as *S. sanctipauli*. Resistance to temephos has since been recorded in all river basins of the OCP area and in almost all the known black fly cytotypes, probably by genetic introgression of genetic resistance alleles among these cytotypes (Boakye & Meredith, 1993).

The appearance of a focus of resistance in the Lower Bandama led OCP to abandon the 'all-temephos' larviciding strategy. It was replaced by an alternate use of several insecticides in order to break the temephos resistance of the black flies. This new strategy resulted in large-scale applications of *B.t.* H-14 during the dry season in the areas of temephos resistance and applications of chlorphoxim during the wet season. But very soon, a resistance to chlorphoxim (an organophosphate, like temephos) was also discovered (July 1981) in the forest species that were already resistant to temephos (Kurtak et al., 1982).

From 1980 to 1997, OCP invested heavily in operational research on insecticides. First of all, it was necessary to select compounds that were cost-effective and not toxic for humans and the non-target aquatic fauna. In addition, it was necessary to optimize their use in relation to the eventual emergence of resistance, while preserving the aquatic environment and maintaining reasonable application costs (Calamari et al., 1998). Therefore, several hundreds of compounds and/or formulations have been evaluated by OCP in an intensive screening programme leading to the selection of seven operational insecticides, six chemical and one biological (*B.t.* H-14) compounds.

Among these alternative insecticides, carbosulfan (introduced in 1985) and permethrin appeared to be promising, provided they would be used only during the rainy season, and for a limited number of applications, in rivers where black fly resistance to temephos occured. By 1991, chlorphoxim was no more commercially available, so it was replaced by two other organophosphates: phoxim and pyraclophos. Later, OCP selected etofenprox, a 'pseudopyrethroid' that was clearly less toxic to fish when compared to permethrin, which was then used as rarely as possible. So, by 1994, seven operational larvicides were available for rotational applications in OCP to control *S. damnosum* s.l. larval populations. Temephos was still widely used in over 80% of the OCP area during the wet season, together with *B.t.* H-14 during the dry season. Because of resistance of the black fly vector, various amounts of these insecticides have been used since the origin of OCP (Table 3), which required an

Table 3. Amounts of insecticides (in liters) used by OCP since its beginning in 1975, to its end in 2002

Pesticides (litres)	Temephos organophosphate	Chlorphoxim organophosphate	B.T. H-14 Biological insecticide	Permethrin pyrethroid	Carbosulfan carbamate	Pyraclofos organophosphate	Phoxim organophosphate	Etofenprox pyrethroid
year								
1975	75 631							
1976	129 947							
1977	155 615							
1978	215 879							
1979	263 377							
1980	184 517	5 713	416					
1981	130 000	70 000	1 500					
1982	162 750	6 699	232 986					
1983	74 807	35 796	310 000					
1984	77 000	57 000	257 000					
1985	130 000	15 000	211 000	3 000	9 000			
1986	895	2 784	385 000	149	20 000			
1987	59 509	29 152	269 803	9 436	7 524			
1988	83 998	79 431	365 321	25 575	11 114			
1989	88 193	64 762	270 752	50 820	30 583	2 556		
1990	108 477	19 713	404 032	44 501	35 586	2 862		
1991	76 819	1 065	271 665	44 546	33 158	31 836	20 281	
1992	47 050		375 954	22 510	27 059	54 566	35 470	
1993	74 795		208 239	19 775	12 666	45 537	19 887	
1994	46 927		224 628	28 438	12 688	43 826	19 785	5 458
1995	40 646		226 158	13 476	19 143	37 049	18 257	16 818
1996	27 450		205 855	20 265	6 100	33 112	23 679	14 362
1997	34 612		194 396	14 596	12 990	26 782	32 482	20 842
1998	22 267		219 984	18 834	7 083	29 382	15 847	14 193
1999	23 744		193 932	17 711	4 784	16 448	12 915	12 005
2000	20 857		226 610	17 583	7 856	26 270	14 878	12 267
2001	9 911		168 629	7 827	1 412	10 505	6 315	9 730
2002	1 927		177 236	7 765		9 426	270	14 256
Total	**2 366 430**	**378 115**	**5 279 325**	**359 040**	**258 746**	**361 386**	**219 880**	**106 303**

adaptation of the operational control strategy (cf. see below).

The monitoring of the organophosphate resistance

In order to monitor the susceptibility of *S. damnosum* s.l. larvae to insecticides, OCP rapidly developed simple and reliable methods. These tests, whose guiding principles were described by Mouchet et al. (1977) for chemical insecticides and Guillet et al. (1985) for *B.t.* H-14, were easily achievable in the field. They helped to determine, for each insecticide, the diagnostic doses that could detect even minor decreases in the susceptibility of the black flies to a given insecticide. These techniques demonstrated considerable improvement concerning the resistance to temephos in the last years of OCP, because the resistance persisted only in the Lower Bandama and Lower-Comoé River in the Côte d'Ivoire, and this on a relatively low level. Similarly, resistance to phoxim (which had replaced chlorphoxim) was not important in the last years of OCP. With regard to pyraclofos, only one case of resistance was reported in the Marahoué River after 16 consecutive weekly applications that were carried out for experimental reasons. This resistance, fortunately, quickly proved reversible in the absence of the selection pressure through this insecticide. To date, no resistance has been detected for the other insecticide families (i.e. the non-organophosphates).

An adaptive operational control strategy

Alongside the programme to screen *Simulium* larvicides, OCP developed a strategy of using these compounds that allowed a break in the resistance of black flies to temephos, chlorphoxim, and to phoxim, as well as to avoid the development of black fly population resistance to other insecticides. Among the possible management strategies of resistance, one consisted in alternating, in time, the insecticides belonging to different families. This rotation enabled the reduction of the insecticide-specific pressure on a given *Simulium* population and, thus, decreased the chances of selection of genes providing resistance in this population. It would have been relatively simple to implement this strategy if all the rotated products had the same characteristics (except the resistance factor) as temephos. Insecticides would then have been limited, and chosen only in terms of the management of resistance, because all other factors such as efficiency, costs, physical properties and toxicity would have no relevance. Unfortunately, these other factors were relevant, which made the implementation of the rotational strategy even more complex (Guillet et al., 1991).

Due to its characteristics, *B.t.* H-14 was the best insecticide to counter resistance to organophosphates. However, its operational dose was relatively high, which limited its use to period with relatively low river discharges. Since 1985, however, the improvement in the commercial formulations made it possible to treat rivers at more elevated discharges of 75–100 m3/s.

Because different insecticides potentially affect different groups or species of nontarget invertebrates (confirmed later by Yaméogo et al., 1991b, 1992; Yaméogo, 1994; Crosa et al., 2001), their successive use could result in a sequential insecticide pressure (and perhaps eradication) on some non-target organisms. Therefore, to reduce environmental impact, strict rules of use were applied for each insecticide. These rules considered in particular the toxicity of the chemical insecticides for the non-target fauna, and limited their use to a maximum number of annual applications and/or discharge levels. For example, permethrin could only be used if the river discharge exceeded 70 m3/s and at maximum 6 times per year on the same river stretch (Hougard et al., 1993).

Difficulties encountered in the implementation of a long-term biomonitoring

Over the long-term, biomonitoring faced various unexpected factors or events that made the interpretation of the results more difficult than was thought at the beginning of OCP. Some of these factors may have been identified at the beginning of the programme but were underestimated. Others, however, could not be predicted. Here, we focus on eight of these factors that interfered considerably with the long-term monitoring in OCP.

(1) The conceptual framework to implement the long-term monitoring used the paradigms in ecology that prevailed in the 1970s, at the inception of OCP (Resh et al., in press). In the 1970s, when the International Biological Programme had already been underway for several years, the ecosystem approach focused on productivity and food webs. At that time, the debate about non-equilibrium versus equilibrium systems was still dominated by the equilibrium proponents (Statzner et al., 2001). Schematically, the view was that ecosystems fluctuate around a mean

Table 4. Main drivers of environmental changes and perceptions that were expected to occur in any long-term biomonitoring programme such as OCP

External large scale drivers
Climatic changes and river discharges
Development of human demography and related pressure on water and rivers resources
Local or regional socio-economic drivers
increased land use and land cover change: siltation, nutrient inputs, etc.
landscape changes and disappearance of gallery forest
increased fisheries pressure
irregular fisheries techniques using pesticides
dam construction and flow regulation
bridge and road constructions
increased industrial pollution from agro-industry and industrialised agriculture
species introduction (e.g. water hyacinth)
likely resistance of non-target fauna to larvicides and other pesticides
OCP constraints
availability of non-harmful larvicides for the non-target fauna
rotational use of insecticides
ecological group recommendations
money restrictions (number of monitoring stations, frequency of sampling)
resistance of the vector to organophospate and use of other insecticides
operational technology of appropriate insecticide dosage
(accurate discharge estimates, application of insectide quantities)
Change in scientific knowledge
poor ecological knowledge of the fauna and rivers systems at the start of OCP, improved through research in the programme area
paradigms of steady state replaced by system dynamics approaches
development of bio-molecular techniques and their use for vector-parasite epidemiology
improved storage and analysis of data
improved knowledge of taxonomy and biology of non target organisms

state, and that any disturbance would be regulated by the system supposed ability to be resilient (Holling, 1973). As a consequence, the monitoring programme was designed to assess how far from the expected steady state the system would be 'pushed' if insecticides had significant impacts. In some way this was an 'impact' approach: what is the deviation compared to the reference situation supposed to prevail at the beginning of OCP? In this context, the idea that the ecosystem would recover after disturbance (i.e. after the period of spraying insecticides) was a clear reductionist approach that did not take into account many of the other possible drivers of ecosystem changes. Thus, it was not really a system approach. In fact, the interpretation of the biomonitoring data had to face the influence of many other changes (see Table 4) that were not assumed to be important at the beginning of OCP. In other words, the monitoring (1) started with a short-term view for a long-term programme and (2) did not appropriately consider the parameters that changed slowly on the short term but became very significant on the long-term (cf. the question of the 'invisible present' brought up by Magnuson et al., 1990). These difficulties relate to time lags, which may occur between cause and effect, i.e. the time required before ecological responses to a disturbance permeate natural systems to the level at which they can be recorded in terms of a significant change.

(2) The situation appeared more complex a few years later, when concepts in general ecology had evolved greatly. While homeostasis and equilibrium states were considered to be the norm in the 1970s,

the ecological thinking afterwards was dominated by concepts of natural disturbance and patch dynamics (Pickett & White, 1985). The idea that short- and long-term variability were key elements in understanding the functioning of ecosystems was conceptually attractive, but was difficult to manage in research programmes at a time when tools like computers were not yet operational. In the OCP area, the existence of large climatic fluctuations had immediate or delayed effects on the aquatic fauna. But how could the dynamics of communities living under such fluctuating constrains be assessed? The assessment of the respective role of external climatic drivers in relation to the possible impact of larvicides thus became a difficult task of the biomonitoring. For example, there was a debate about the interpretation of fish monitoring data illustrating the dramatic disappearance of the fish species *Schilbe mystus* at several monitoring stations in the 1970s. Was it the result of larviciding or the result of long-term trends in the population dynamics? Later, the reappearance of that species at most monitoring stations confirmed that population fluctuations were primarily linked to hydrological inter-annual changes and not to larvicide treatments.

(3) Human impacts on the monitored rivers led to other unexpected situations such as bridge construction that altered the monitoring stations. Dams were also built during the OCP on several of the monitored rivers, including the Sassandra, the Bandama, and the Volta. The consequences of these human impacts on habitats, water quality, and riverine fauna have not been accurately recorded but seemed to be so obvious at some monitoring stations that they were abandoned or moved to other river sections.

(4) During the period of OCP, the fishing pressure on the rivers considerably increased because migrant fishermen moved quickly to other places when the fish stocks were depleted in a river stretch. It was also reported that insecticides had been used as fish poisons at many occasions in various places of the OCP area.

(5) Another unexpected consequence of the success of OCP in controlling *Simulium* has been the human recolonisation of onchocerciasis-freed valleys. The resettlements along the rivers led to changes in land cover and land uses, which changed the riparian habitats (cf. above), and the leaching of pesticides and nutrients used in agriculture led to some eutrophication of the rivers

(6) In different places of OCP area, agro-industrial complex developed since the beginning of OCP. In some cases, heavy organic pollution of rivers resulting from agriculture wastes have been reported.

(7) Insecticide resistance of non-target invertebrates likely occurred, similarly to the vector resistance described above. However, this issue has never been investigated in the OCP area, and probably not in other aquatic monitoring programmes. It was probably a source of bias for long-term monitoring given that the responses of animals as bio-indicators of environmental changes (or insecticides in the case of OCP) were modified over time.

(8) More recently, parts of the monitored rivers have been invaded by the pest plant *Echhiornia crassipes* (water hyacinth) which likely changed the environmental conditions of the aquatic non-target fauna.

Obviously, all the above factors had consequences on the functioning of the river systems and modified the populations dynamics of the non target fauna. To what extent did this occur? That is quite difficult to assess.

The need for research to understand the impact of long-term larviciding

At the beginning of OCP, apart from systematic inventories, the knowledge of West African invertebrates was almost non-existent and that of fish was very poor (Resh et al., in press). The few biological fish studies available at that time (e.g. Daget, 1957) indicated that many species spawn only once during the flood season, and exhibit both dry season movements within the main channel, and upstream migrations just before flooding to reach floodplain spawning areas. Some information was also available on the impact of large dams on the riverine fauna (Lelek & El Zarka, 1973; Petr, 1986). However the most important research programme on West African freshwater ecosystems at that time had been conducted in the Lake Chad basin, where the fish and the invertebrate fauna were studied for many years (Carmouze et al., 1983). Of particular importance were the studies of changes in the community structure in relation to inter-annnual climatic variability. These results from the lake Chad basin helped to explain the monitoring data over the long-term of OCP.

Temporal dynamics of invertebrates

The knowledge of the turnover time of the species is a critical topic to understand the dynamics of communit-

Table 5. Approximate duration (in weeks) of the aquatic phase (which varies among seasons) of the life cycle of benthic insects in various river reaches in the OCP-area (after Statzner, 1982; Wuillot, 1991; Schorscher, 1992)

Taxon	Duration (weeks)
Baetid mayflies	
Afrobaetodes sp. 1	2–5
Afroptilum sp. 1	5–15
Afroptilum sp. 3	1–5
Afroptilum sp. 4	1–2
Afroptilum sp. 5	2–3
Afroptilum sp. 6	6–9
Baetis sp. 1	1–4
Centroptiloides spp.	4–8
Ophelmatostoma camerounense (Ulmer)	1–4
Pseudopannota bertrandi (Demoulin)	1–5
Pseudopannota muganinani Elouard & Gillies	2–7
Hydropsychid caddisflies	
Aethaloptera dispar Brauer	≥ 8
Amphipsyche senegalensis (Brauer)	8–12[1]
Cheumatopsyche copiosa Kimmins	4–8[1]
Cheumatopsyche digitata (Mosely)	6–12[1]
Cheumatopsyche falcifera (Ulmer)	4–8[1]
Macrostemum distinctum (Ulmer)	9
Libellulid dragonflies	
Zygonyx spp.	≥ 8

[1] Few larvae in populations of these species would have survived the dry season (i.e. a period of flow cessation) in the hyporheos or in stagnant pools, and thus should have a longer aquatic life.

ies submitted to weekly treatments. However, very little information on invertebrate life cycles existed for the tropics when OCP began, and it was only assumed that some lotic insect species need several months to grow to full size (Hynes, 1970). Therefore, in the OCP-area, some species would perhaps be exposed to many of the weekly insecticide treatments during their life span.

After OCP began, all quantitative studies of West African lotic macroinvertebrates that covered an entire hydrological cycle demonstrated clear effects of the hydrological dynamics on the benthic densities of instars of species, species, genera, or families; this was the case in temporary and permanent river sections as well as in natural or treated river sections (Hynes, 1975; Statzner, 1982; Elouard, 1983; Schorscher, 1992). After flow resumed in temporary rivers, recolonization by insects was usually by oviposition of aerial females (Hynes, 1975; Schorscher, 1992). Only within the hydropsychids, older larvae or pupae of some species reappeared on rapids shortly after the flow resumption. Thus, some larvae survived several months of flow cessation, either deep within the substrate (i.e. the hyporheos) of rapids or in the remaining stagnant pools (Statzner, 1982; Schorscher, 1992). After flow resumed, taxa with aerial re-colonization subsequently appeared, and it took 2–3 months until a diverse benthic fauna was established (Hynes, 1975; Schorscher, 1992). In permanently flowing, untreated river sections, only few species (e.g. *Tricorythus* sp., *Simulium unicornutum*) were missing for longer periods (7–9 months), whereas most species occurred throughout the year (Elouard, 1983). However, these latter species had peak densities during either one (flood, ebbing of flood, low flow, rising flood) or two hydrological periods (Elouard, 1983).

After flow resumption, the re-colonization of a temporary stream by insects suggested that insect life cycles had a duration of approximately 2 1/2 months (Hynes 1975). Subsequent field analyses of the instar composition at natural sites or at sites previously treated with insecticides provided information about the generation time of several species of insects, which varied from 1 to 15 weeks (Table 5).

During the OCP, it had been confirmed or discovered that many of the taxa having detailed information on their life cycles were predators of the vector (Service & Elouard, 1980; De Moor, 1991, 1992; Schorscher, 1991, 1992; our Tables 1 and 2). Particularly the hydropsychids, a very abundant group in the OCP-area (Petr, 1970; Hynes, 1975; Statzner, 1982; Elouard, 1983; Schorscher, 1992) had species that predated considerable numbers of the vector's larvae (De Moor, 1991; Schorscher, 1992) and, at the same time, had considerably longer generation times than the vector. Therefore, hydropsychid species were potentially exposed to 4–12 of the weekly insecticide treatments during their aquatic life.

Longitudinal zonation of rivers

The basic idea of river zonation is that the aquatic fauna respond to large-scale gradients such as the unidirectional flow and increasing discharge of water, but also the increasing habitat heterogeneity from the source to the mouth of a river. This basic pattern of river systems may be modified by local, geological, or geomorphological features.

Among the many attempts to propose a model of biological zonation in rivers, Ilies & Botosaneanu (1963) used faunistic criteria to divide the river course into headwater (or creon), middle reach (or rhitron), and lowland (or potamon) zones. In contrasts, the River Continuum Concept (RCC) (Vannote et al., 1980) provided a conceptual, functional framework of river zonation, by focusing on changes in trophic resources with increasing river size. Briefly, the RCC suggests that the relative abundances of various food types vary predictably with stream size, and that the relative abundances of consumer guilds are correlated with those of their major food resources.

The longitudinal zonation of insects in the OCP-area was totally different from patterns found elsewhere in the world (Gibon & Statzner, 1985). In general, no clear species replacement occurred along West African rivers. Instead, species richness steadily increased downstream because of the occurrence of additional species. This distribution pattern was related to the increase in the length of the period without flow from the temporary headwaters to the permanently flowing lower-river sections, the consequent lack of well-defined spring sources, and the gentle slope of the channels. Thereby, many rivers in West Africa lack distinct downstream changes in the hydraulic conditions, which produce the typical zonation patterns of invertebrates elsewhere in the world (Statzner & Higler, 1986). As a result, nontarget insect species of the treated rivers generally occurred also in sporadically or never treated smaller tributaries within the OCP-area, as well as in untreated river sections outside the OCP-area (Gibon & Statzner, 1985). In addition, abundant species of the insect communities in treated rivers are usually distributed across large areas (Durand & Lévêque, 1980, 1981).

The distribution of fish along a longitudinal gradient has been studied in the Bandama basin (Mérona, 1981; Lévêque et al., 1983), the Mono (Paugy & Bénech, 1989), and the Upper Niger (Hugueny, 1990). In the Bandama basin, three main zones were distinguished (Lévêque et al., 1983): (1) headwaters and small tributaries, which are temporary streams running for part of the year, but dry out or eventually remain as pools during the dry season; (2) a long, relatively uniform middle reach, which however had several successive alternations between slow flowing and rapid reaches; and (3) a relatively short estuarine zone where saline waters may penetrate a few tens of kilometres upstream. The low headwater streams in the Bandama basin were usually inhabited by a small number of fish species that had a small adult size and short life-span (small *Barbus*, Cyprinodontidae, small Characidae) (Mérona, 1981). In the long middle reach, large species such as *Alestes baremoze* (de Joannis), *Brycinus nurse* (Rüppel), *B. macrolepidotus* Valenciennes, *Hydrocynus forskalii* (Cuvier), and *Schilbe mandibularis* (Günther) were characteristic of the calm and deep reaches. Fish assemblages in the riffles were mostly comprised of small species adapted to live in a turbulent environment (e.g. *Amphilius*, *Phractura*) or juveniles of larger species (*Labeo parvus* Boulenger, *Synodontis bastiani* Daget), as well as species inhabiting rocky habitats where they find shelter in crevices (*Afromastacembelus*, small mormyrids). The existence of a long mid-course with little large-scale physical change was also observed in the Ogun River in Nigeria (Sydenham, 1977). A peculiar zonation pattern was observed for a few species of fish (*Brycinus longipinnis* (Günther) and *Hepsetus odoe* (Bloch)), which occurred in the upper reaches, disappeared in the mid-course zone where they were replaced by other species (*Brycinus nurse*, *B. imberi* (Peters), *Hydrocynus forskalii*), and reappeared in the estuarine zone.

In the context of the RCC, Lowe-McConnell (1987) suggested some general trends for fish, such as a higher abundance of surface-eating insectivores and omnivores that consume riparian allochtonous material in the upper course of rivers, or the presence of herbivores and benthic detritivores in the lower course. Such a distribution was found in the Niandan River (Hugueny, 1990) and the Mono River (Paugy & Bénech, 1989), but it has not been demonstrated that it was clearly related to changes in food availability.

Response of fish communities to inter-annual changes in flow regimes

The OCP area encompassed a large number of river systems. Details of hydrological and chemical characteristics of many rivers are available in Iltis & Lévêque (1982) and Mahé (1993). Most of rivers are situated in the savanna and have a tropical hydrological regime, characterised by a flood period from June–July to December, with a peak in November. During floods, the rivers overspill from their main channel, inundating the fringing plains. Rivers discharge is very low during the dry season and the upper course is sometimes intermittant and reduced to a series of pools.

The volume and length of flood is directly related to the distribution and abundance of rainfall and, as a result, of climatic fluctuations; therefore the water discharge exhibits large year-to-year changes. The occurrence of wet and dry years however is not randomly distributed in time; the drought that affected West Africa since 1968 has resulted in a series of below average floods during the last two decades. The reduction of flooding as a result of natural climatic changes, such as the severe Sahelian drought of 1970–1977, had important consequences for fish biology and floodplain fisheries. For example, the population of *Alestes baremoze* decreased dramatically in the Chari River and southern Lake Chad (Bénech et al., 1983) as a result of the strongly reduced flooding of the North Cameroon Yaéré floodplains, where the young of that species normally spend their juvenile phase.

The relationship between flood intensity and duration, and fisheries production, is well known (Welcomme, 1979). In a study of the Logone floodplain (North Cameroon), Bénech & Quensière (1983) found a positive correlation between fisheries production and flood volume during the severe Sahelian drought of 1970–1977. For different fish species, they also observed a significant correlation between growth of juveniles and flood intensity. Dansoko et al. (1976) also found that juvenile growth in two species of *Hydrocynus* was poor during two years of low floods in the inner delta of the Niger.

In a more recent study on the effects of hydrology on the evolution of the fisheries of the inner delta of the Niger River from 1966 to 1989, Lae (1992, 1994) found a high correlation between the annual catch and the flood intensity for the same year. It appears that competition for food resources is not a serious factor in determining the composition and abundance of African riverine fish communities in the main channel of large rivers, i.e. the potamon (Welcomme, 1989; Lae, 1995). Rather, it seems that limitations in the extent of breeding areas caused by a lack of floods during the drought period play a greater role in determining the relative abundance of species. For example, in the inner delta of the Niger, fish catches for the period 1969–1991 were characterised by a depletion of species such as *Gymnarchus niloticus* Cuvier and *Polypterus senegalus* Cuvier, whose reproduction is linked to the floodplains. In contrast, families such as the Cichlidae and Clariidae increased to constitute 30% and 20%, respectively, of total annual catches of fish during the drought period (Lae, 1995).

The way in which a fish community reflects the 'hydrological memory' of a system also depends on its age structure. When most of the community is composed of long lived species, many year classes are present and this type of age structure tends to average out the hydrological regimes of several years and to reflect only long-term trends. Conversely, when the fish community consists of few age classes, it will be highly correlated with flood intensity in previous years (Welcomme, 1986). Actually, most of the fish species in African rivers fit this latter pattern because they have a relatively short life that does not last more than a few years.

Thus, West African riverine fish respond clearly to the dynamics of flow regimes. It is obvious that these natural dynamic interfered with the long-term biomonitoring of fish, as the monitored rivers had considerable discharge variations during the period of OCP (Fig. 4).

The results of 30 years aquatic biomonitoring: temporal dynamics of fish and invertebrates

The major concern of OCP in terms of the aquatic environments exposed to insecticides to control populations of *Simulium* has been to avoid long-term or lasting changes in aquatic biodiversity. The major groups of organisms that have been monitored during almost 30 years of OCP operations are fishes and non-target invertebrates (cf above).

Fish monitoring

Potential impacts of larviciding on fish have been evaluated by regular assessments of changes in species richness per catch, catch per unit effort (CPUE) of fishing, and the coefficient of condition of selected, abundant fish species (Lévêque et al., 1988; Paugy et al., 1999). Bioaccumulation of insecticides was also studied but only sporadically (e.g. Matthiessen & Johnson, 1978).

Species richness of experimental catches (the number of species caught in a standard set of experimental gill nets during two nights), showed different long-term trends observed in three major areas of the Programme (i.e. rivers in Côte d'Ivoire, Volta basin rivers in Ghana, and Niger basin rivers in Guinea) (Fig. 5). After a period of species richness decline, especially in rivers of Côte d'Ivoire and Volta basin, recovery and improvements in species richness has been observed since 1994 and 1996 in all rivers. Thus, after many years of larviciding with several insecticides, there is no evidence that a reduction of fish species diversity in treated rivers occurred. A similar observation was made after the initial 10 years of monitoring during

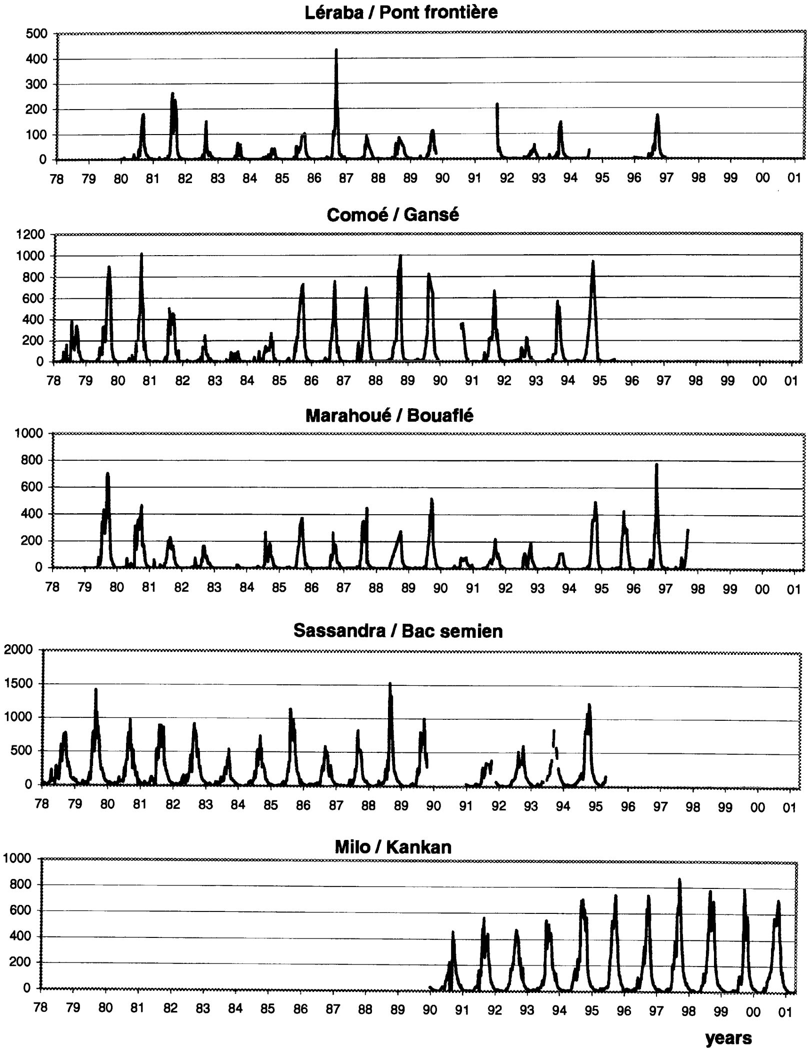

Figure 4. Long-term changes in the annual discharge for some of the monitored rivers. Low discharges were characteristic of the early 80s and the early 90s in many rivers of the OCP area.

which only three larvicides had been used. Comparison of species richness changes with hydrological trends suggest that the observed trends in species richness might be attributed to climatic factors and the long period of drought that occurred for many years in West Africa.

Overall trends of catch per unit effort (CPUE) in relation to larviciding during 20 years of fish monitoring also indicate different scenarios in the different major basins of the OCP area, and sometimes among rivers of the same basin despite a generally similar larviciding regime (Fig. 6). For example, catches were reduced in Côte d'Ivoire rivers between 1989 and 1993, but increases were observed in all rivers since 1995, even though larviciding was stopped on different rivers at different times. In the Niger basin, no decreases in CPUE catch have been observed since monitoring began, while increases beyond the initial

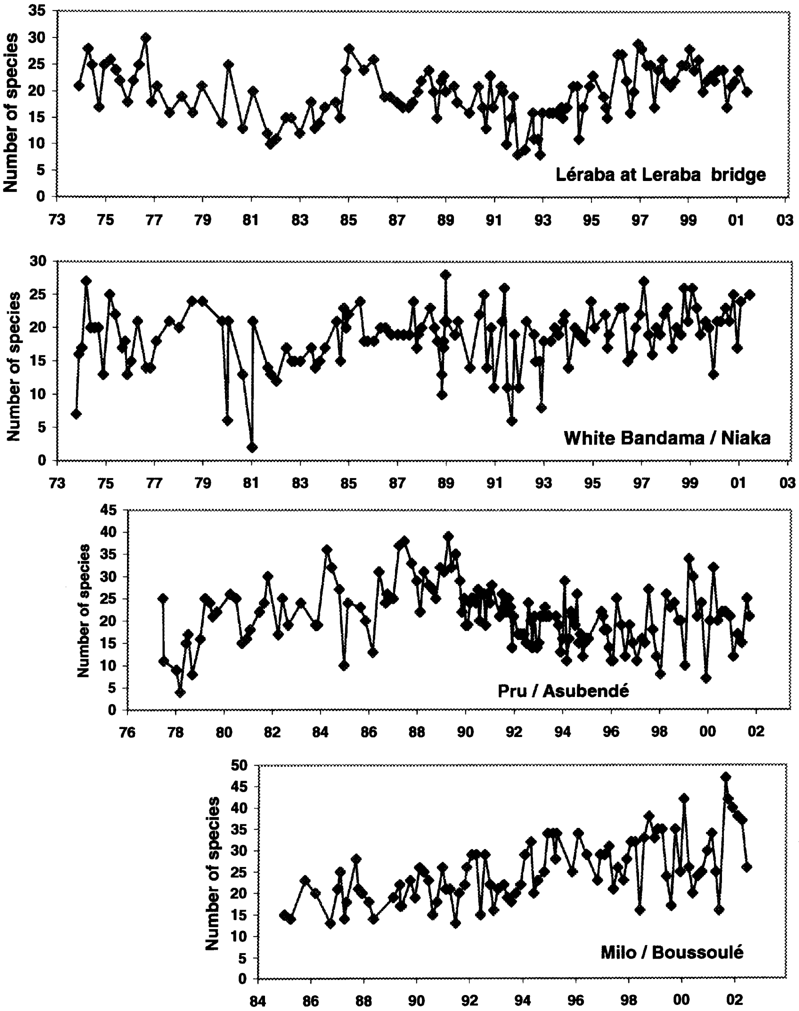

Figure 5. Long-term changes in fish species richness per sample in some monitored rivers.

CPUEs have been observed in the three rivers monitored in the basin since 1994. In the Volta basin, different trends in CPUE have been observed over the years. However, a common seasonal pattern of high CPUE at low water periods (December/January till April/May) and low CPUE at high water periods (July/August till October/November) was observed for all basins and rivers, illustrating again the dominant role of the discharge regime.

The coefficient of condition (a ratio between weight and length) expresses the 'well being' of fish, so it to assessed direct effects of larvicides on fish (acute toxicity) and/or indirect effects through larvicide impacts on their food sources, which are in many cases aquatic invertebrates. Over the years, various assessments of trends in this coefficient of several fish species in the OCP area indicated only fluctuations around expected means, but no significant changes in values. This led to the conclusion that larviciding had not directly affected fish, suggesting that if fish food items had been affected, other types of food were used instead.

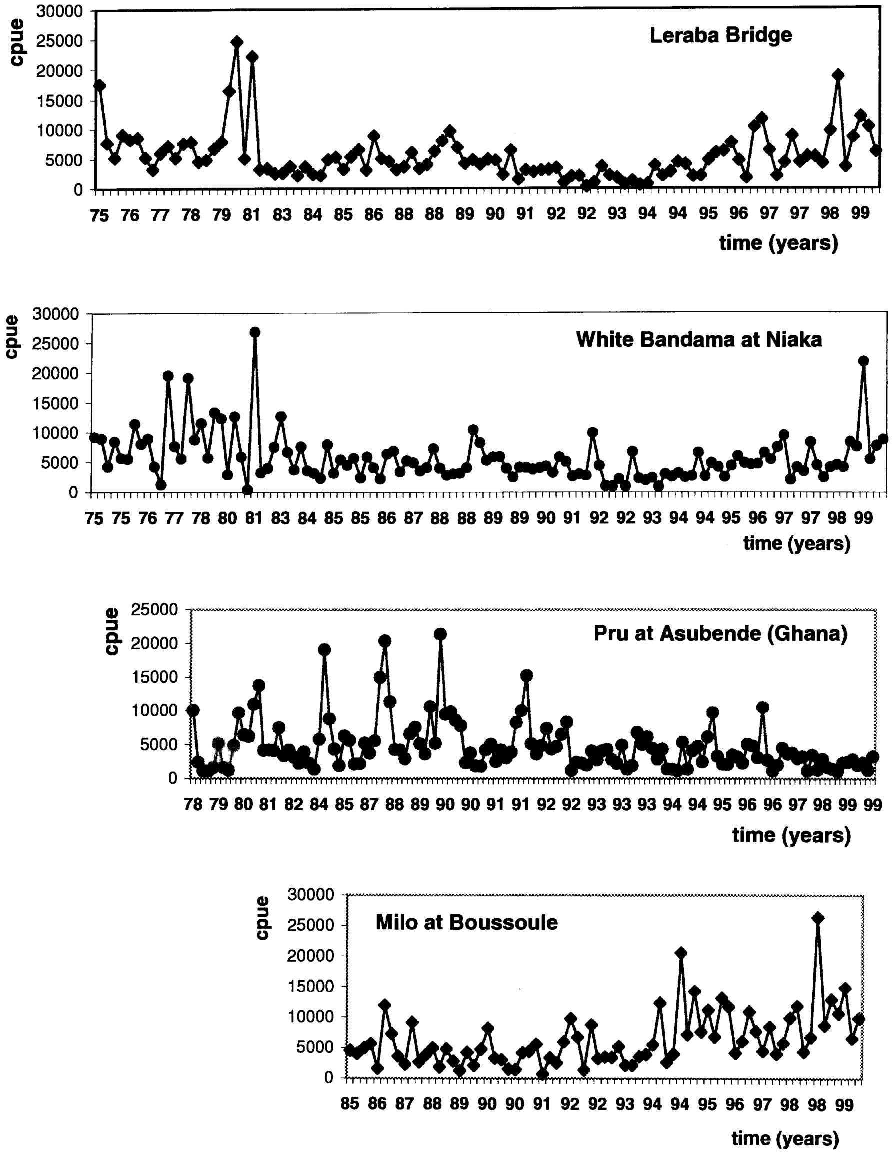

Figure 6. Long-term changes in fish catch per unit effort (CPUE) per sample in some monitored rivers.

Bioaccumulation of pesticides in fish was a major concern in OCP as a result of the widespread concern about DDT. Actually, the effects of organophospates in laboratory experiments showed that fish were able to accumulate temephos (Matthiessen & Johnson, 1978) but that this accumulation seemed to be limited and did not increase to a point observed for DDT. Field data from the OCP area showed that temephos did not accumulate in fish (Quélennec et al., 1977). Moreover, in the field conditions, the acetylcholinesterase activity in fish brain tissue was not significantly different between rivers treated with temephos and untreated rivers (Antwi, 1985).

Invertebrates monitoring

The specific concern of OCP about larviciding effects on non-target invertebrates had been to prevent loss of faunal diversity and to maintain the quality of biomass available for use by higher trophic levels of the aquatic ecosystem food web (cf above).

Field impact assessment of larvicides evaluated two types of data (Surber samples and day and night drift collections) (Crosa et al., 1998). In analyzing invertebrate data that were collected using various sampling strategies between 1977 and 1996 (Yaméogo et al., 2001b) evaluated long-term changes of invertebrate abundances (cf. Fig. 7) (usually grouped together at the family level) considering taxonomic composition as well as trophic structures. Because Surber samples provide a qualitative and quantitative assessment of the invertebrate community at the sampling location, they provide a clear way to examine the community changes in terms of taxonomic and functional structure. Generally, the results indicated that different larvicides had different impacts in different rivers and on various groups of invertebrates. The greatest reduction in the diversity and abundance of the invertebrate assemblages occurred during phoxim, permethrin, carbosulfan, and pyraclofos treatments (Yaméogo et al., 1992). Temephos and *Bt* H-14 were the least stressful larvicides. The taxonomic units that exhibited clear changes in their abundance were Tricorythidae, Leptoceridae, Chironomidae, and Baetidae.

Concerning their functional structure, all invertebrates communities were dominated by gathering collectors and, to a lesser extent, by filtering collectors. The abundance of these functional feeding-groups provided evidence about the abundant availability of fine particulate organic matter that characterised the food resources within the studied rivers. The abundance of these two trophic groups tended to increase during the application of all insecticides but *Bt* H-14 (Yaméogo et al., 2001b).

Synthesis

From the previous two sections, it can be concluded that the effect of insecticides on the nontarget aquatic fauna was usually low for fish, whereas results for invertebrates indicated changes in the taxa composition and community structure even at the family level. However, Yaméogo et al. (2001b) concluded that these changes were ecologically acceptable as the analysis of invertebrate feeding groups indicated that the general functioning of the aquatic systems was not clearly affected. To be sure that there were not irreversible loss of taxa, the question of recovery of the aquatic fauna was raised by the Ecological Group. Did the aquatic community return to a structure and taxa composition at the end of the treatment period that is more or less similar to that of the pre-treatment period? Recent field data indicated that recolonisation by taxa that were affected during the treatment period (for example, the stonefly *Neoperla* sp. and the shrimp *Caridina* sp.) were observed at a majority of stations after larviciding stopped. The ability of the aquatic invertebrate fauna to recover in previously treated rivers has therefore been demonstrated. In addition, Resh et al. (in press) reviewed additional research studies that indicated that the recovery potential of non-target insects in the OCP-area was high.

Conclusions

From an epidemiological aspect, the goal of OCP has been achieved: onchocerciasis has been virtually eliminated from the OCP-area as a disease of public health importance, and as an obstacle to socio-economic development (WHO, 2002). The question now is how to maintain an efficient surveillance and control of the parasite in the onchocerciasis-freed areas in order to avoid a return of the disease.

From the environmental perspective, this programme greatly contributed to improvements about our knowledge of the biodiversity and ecology of West African rivers. Actually, among public health programmes, OCP had a unique position for its long-term consideration of the protection of the environment. Thanks to OCP we have now: (1) a better knowledge of the fauna and ecology of West African rivers, particularly for insects and fish (Lévêque et al., 1990, 1992; Paugy, 2002); (2) a better knowledge of the long-term dynamics of aquatic populations in relation to climatic changes and human influences; (3) abundant information about the sensitivity of the African aquatic fauna to diverse chemical products; and (4) an exceptional data-base compiled of all the results recorded during the environmental monitoring programme, which is managed and available at the OCP headquarters. Obviously, all this knowledge will be useful for other activities in relation to socio-economic develop-

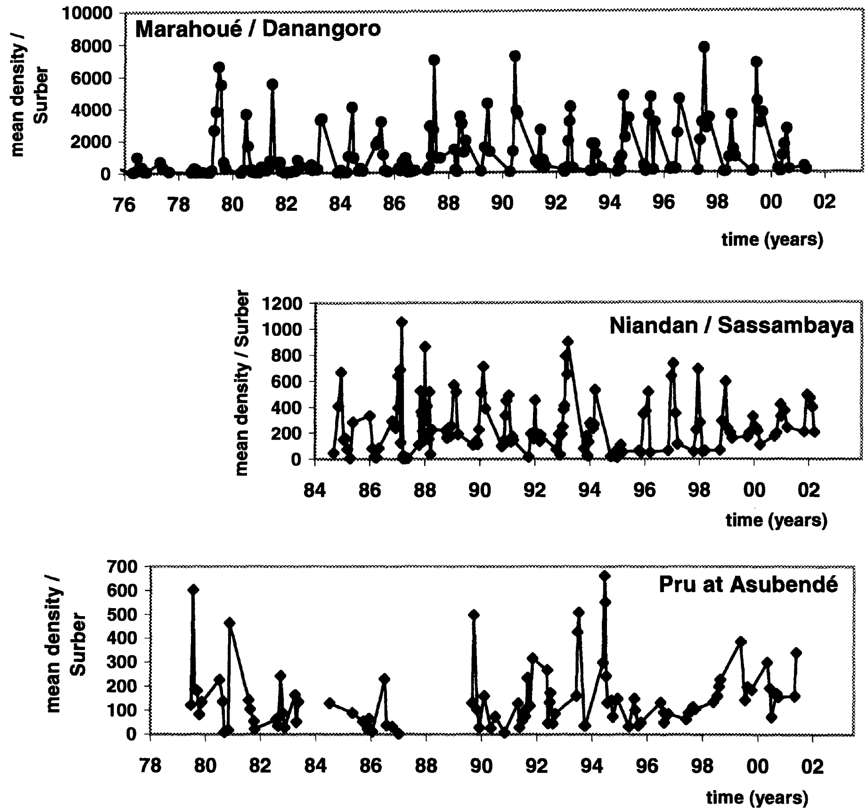

Figure 7. Long-term changes in the density of invertebrates on the rocks, as estimated using Surber samplers, in selected monitoring stations.

ments, and for the conservation of the West African environment.

However, at the end of OCP in December 2002, some major questions concerning the consequences for the environment of the control of river blindness in West Africa remain, even if their nature has changed over the past 30 years. For example, what will be the environmental effects of the large resettlements that continue to occur in the once abandoned and now onchocerciasis-freed valleys? It would be tragic if the efforts made in protecting the aquatic environment and its freshwater biodiversity during OCP be of little use, given the massive land changes that are likely to occur with unplanned, unsustainable resettlement policies? Agricultural pesticides are also an environmental problem when they are use for fishing. Although such techniques are not allowed and may create risks for human health, recently it has been reported to occur repeatedly in the OCP area. It is difficult to quantify the impact of such practices but probably, in synergy with other threats, they may deplete fish populations that are already heavily fished, as well as invertebrate populations present.

Another potential threat to aquatic biodiversity is the recovery of *S. damnosum* populations, which is another biodiversity issue (e.g. recovery after selection pressure ceases). After being controlled for many years, the black flies have reappeared, when larviciding ceased, in the areas where the disease was under control. In the early 1990s, the black fly bite rate reached again high levels in some areas of OCP (Hougard et al., 1998). The return of black flies, while no longer transmitting onchocerciasis, was perceived by the human population as a major nuisance. To ameliorate this public concern, OCP encouraged individual actions against black flies, through low-cost control techniques such as ground application of insecticides to local breeding sites. Two insecticides (*Bt* H-14 and temephos) have been recommended and OCP has provided these products for use by local human communities. However, larvicide applications performed by non-specialists for an indeterminate period could create an enormous risk of environmental pollution. Another risk is that local human communities will use the insecticides that are locally available with the danger of causing resistance among the black flies and contaminating the environment.

These topics illustrate the other side of the biodiversity issue: the conservation of aquatic biodiversity must deal with human welfare. In no way, can we accept that human populations must live in an environment that is unhealthy. Moreover, the vector control of the major water-borne and riparian nuisances, for instance black flies, tse-tse flies, tabanids, and mosquitoes, has equal priority in Africa and in temperate countries. In other words, we must develop a realistic approach for situations where human welfare does not enable the full protection of aquatic biodiversity. Conservationists should keep in mind that many wetlands in the world, including those in Europe, have been artificially dried up to prevent malaria. Thus, it is clearly unacceptable to expose human populations to the stress of diseases and nuisances for the sake of conservation. On the other hand, it is not acceptable to destroy the biodiversity, both for ethical and economic considerations, because biodiversity is a major source of goods and services for human societies. Even today, the scientific community has conflicting attitudes about what is 'ecologically acceptable', whereas the stakeholders – those most affected – are usually neither concerned nor informed about the consequences of a biodiversity loss. Obviously, the dialogue among the different socio-economic perspectives has to be continued, which is probably the most difficult issue to tackle. In the case of OCP, the reductionist approach (one goal, one programme) (McMillan & Meltzer, 1996) was adopted instead of a system approach. The Committee of Sponsoring Agencies (WHO, FAO, UNDP, World Bank), in charge of ensuring coordination between the cosponsors of OCP, today faces difficulties in coordinating the various currently developing programmes in the former OCP area. If this co-ordination fails, an integrated ecosystem management that is likely to achieve sustainable development is still far from becoming a reality in West Africa.

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Evolution of the tribe Tropheini from Lake Tanganyika: synchronized explosive speciation producing multiple evolutionary parallelism

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Abstract

One of the most surprising outcomes of recent molecular studies on cichlid fishes of the three Great East African Lakes Victoria, Malawi and Tanganyika, was the stunning rapidity of speciation and cladogenesis at early stages of adaptive radiation. Despite their rapid pace, speciation events were so far intuitively assumed to proceed in a bifurcating and tree-like fashion, even if they could not be resolved by gene phylogenies due to a lack of resolution. On the basis of phylogenetic analyses of the Tropheini, a lineage of endemic rock-dwelling cichlid fishes from Lake Tanganyika, we suggest a pathway of explosive speciation that accounts for a non-bifurcating manner of cladogenesis. This pattern is likely to be the result of the contemporaneous origin of a multitude of founder populations in geographically isolated rock habitats among which gene flow was interrupted simultaneously by a major change of the lake habitat in the form of a rapid rise of the lake level. As a consequence, all new species arising from that vicariance event must exhibit almost equal genetic distances to each other, within the scope of genetic diversity of the founder population(s), even if the actual processes of subsequent speciation and eco-morphological diversification followed independent routes. Our phylogeny also suggests a high frequency of parallel evolution of equivalent trophic specialization in the Tropheini. This phenomenon seems to be an inherent feature of this pathway of speciation, due to the action of similar selective forces on the same set of species colonizing isolated habitats of the same type. Explosive speciation via synchronization of genetic divergence triggered by rapid environmental changes seems to be particularly likely to occur at advanced stages of adaptive radiation, when species are already adapted to particular habitats and have a reduced ability for dispersal.

Introduction

The cichlid fishes of the Great Lakes in the East African rift valley represent a prime model system for the study of adaptive radiation (Fryer & Iles, 1972; Mayr, 1984; Greenwood, 1984; Coulter et al., 1986; Rossiter, 1995; Sturmbauer, 1998; Kornfield & Smith, 2000). Since the first reports on African cichlid species flocks appeared (Boulenger, 1898), many scientists have entered this area of research and substantial knowledge has accumulated in the fields of geology, climatology, limnology, as well as ecology, comparative morphology, behavioral sciences and taxonomy. Important insights concerning key innovations (Fryer & Iles, 1972; Liem, 1973), alternative modes of speciation (Rensch, 1933; Kosswig, 1947; Brooks, 1950; Schliewen et al., 1994; Turner, 1994; Danley & Kocher, 2001; Salzburger et al., 2002a), the role of sexual selection in speciation (Seehausen et al., 1997; Galis & Metz, 1998), and the occurrence of convergent evolution have been gained from these model systems (Kocher et al., 1993; Rüber et al., 1999).

Adaptive radiation is thought to be driven by two types of modulators: intrinsic factors in anatomy, ecology or behavior supplying the potential, and external events providing the opportunity, for radiation. An outstanding feature of the African Great Lakes – viewed as an important external mainspring to promote cichlid radiations (Coulter, 1994; Sturmbauer, 1998; Kornfield & Smith, 2000; Sturmbauer et al., 2001) – are water level changes, caused by variations in rainfall, temperature and evaporation, and for some lakes tectonic activity (Scholz & Rosendahl, 1988; Tiercelin & Modeguer, 1991; Cohen et al., 1993; Johnson et al., 1996; Lezzar et al., 1996; Cohen et al., 1997). For example, the 1997 El Niño event resulted in a marked increase of rainfall in East Africa causing Lake Tanganyika to rise by 2 m within 6 months. Any rise of the lake level may promote population subdivision and the colonization of new habitats by shifting the shoreline according to the basin structure of the lake. The degree of habitat change enforced by such water level fluctuations ranges from small-scale effects to major events that affect species communities throughout a lake simultaneously. In this way, lake level fluctuations may induce cycles of concerted allopatric speciation. Wherever the distance among suitable habitats exceeds the dispersal ability of a species, gene flow is interrupted, genetic differences will accumulate independently and lineage sorting will proceed to ultimately lead to the formation of new species.

The cichlid faunas of African lakes are subdivided into communities specialized to particular habitat types (Fryer & Iles, 1972; Coulter, 1994). Coexistence of a great number of species is possible by sometimes small differences in trophic specialization promoting effective resource partitioning (Hori, 1991; Sturmbauer et al., 1992; Genner et al., 1999), at least during critical periods of time when resources are scarce (McKaye & Marsh, 1983). Furthermore, eco-morphological and behavioral specialization to particular types of habitats such as rocky shores and sand bays affects the dispersal ability. More specialized species become isolated to a higher degree than less stenotopic and thus more mobile species. With 24 currently described species the endemic Tanganyikan tribe Tropheini represents such a highly diverse assemblage of coastal fishes (Poll, 1986). The tribe's actual species number is probably much higher since almost all circumlacustrine taxa represent arrays of sister species (Sturmbauer & Meyer, 1992; Snoeks et al., 1994; Rüber et al., 1999; Baric et al., 2003). Most species of the Tropheini are epilithic algae feeders, specialized to feed on filamentous or unicellular algae, as well as on detritus. They have evolved spectacular ways to deal with food uptake, and marked differences in the pharyngeal anatomy and dentition served as basis for their subdivision at the generic level (Boulenger, 1898; Yamaoka, 1983; Poll, 1986). For example, all species assigned to the genus *Petrochromis* have elongated tricuspid teeth as adaptation to comb unicellular algae and detritus from the rock surface. Likewise, all members of the genus *Tropheus* possess an outer row of bicuspid teeth with a continuous cutting edge and curved conical teeth on the sides of the premaxillary bone (Poll, 1986). Due to their strict specialization to particular niches on rocky habitats most species have a limited ability for dispersal over ecological barriers (Brichard, 1978; Sturmbauer & Dallinger, 1995). This is reflected in the presence of numerous distinctly colored populations and/or sister species. Sister group of the Tropheini are haplochromine cichlids of the genus *Astatotilapia* (Sturmbauer & Meyer, 1993; Salzburger et al., 2002b), which live in swampy zones of Lake Tanganyika, as well as in surrounding rivers and lakes (Poll, 1986). Comparisons of average genetic distances to other lineages of cichlid fishes in Lake Tanganyika suggest that the Tropheini have evolved at a later stage of the radiation than many other tribes (Sturmbauer & Meyer, 1992, 1993; Sturmbauer et al., 1994, 1997). Their age, however, exceeds that of all *mbuna*- and *utaka*-species of Lake Malawi (Sturmbauer & Meyer, 1992; Sturmbauer et al., 2001), and the occurrence of incomplete lineage sorting at the species level has not been observed in all the Lake Tanganyika cichlid species analyzed so far. However, the phenomenon of ancient incomplete lineage sorting, as recently described by Takahashi et al. (2001), might have taken place at the onset of the radiation of the Tropheini.

We used a molecular approach to study the pathway of diversification of this lineage of cichlid fishes within a complex species flock at an advanced stage of radiation. We tested for the strength of the phylogenetic signal in all ancestral branchings of the Tropheini radiation and analyzed the possibility of contemporaneous cladogenesis. We also addressed the phenomenon of parallel evolution of species having equivalent trophic specialization (see e.g. Kocher et al., 1993; Verheyen et al., 1996; Rüber et al., 1998, 1999; Baric & Sturmbauer, 1999; Hanel & Sturmbauer, 2000).

Materials and methods

We analyzed DNA sequences of two mitochondrial gene segments (402 bp of cytochrome *b*; and 385 bp of the control region) of a total of 38 specimens of nine genera of the Tropheini. *Astatotilapia burtoni*, which was identified as the sister group of the Tropheini (Sturmbauer & Meyer, 1993; Salzburger et al., 2002b), and *Haplochromis obesus* were used as outgroup (Table 1). We also included two specimens of the haplochromine cichlid *Ctenochromis horei* in our analyses, since it was tentatively placed within the Tropheini in earlier studies (see also Nishida, 1997), and further two specimens of the limnochromine cichlid *Gnathochromis pfefferi*, which was already shown to branch within the Tropheini (Kocher et al., 1995; Salzburger et al., 2002b). Voucher specimens are deposited at the Royal Museum for Central Africa in Tervuren, Belgium, under the numbers listed in Table 1, or are available from the authors. DNA-extraction, PCR-amplification, and automatic DNA-sequencing were performed according to standard methods (see Salzburger et al., 2002b) using published primers for both gene segments (Kocher et al., 1989; Salzburger et al., 2002b). DNA sequences were aligned by Clustal W (Thompson et al., 1994) and alignments were further increased by eye in the case of the control region. Phylogenetic analyses were performed in three steps. First, the strength of the phylogenetic signal was evaluated for the whole data set. Then phylogenetic trees were constructed using three alternative algorithms, and finally the resulting topologies were statistically evaluated. The different topologies were compared by the nonparametric two-tailed Wilcoxon signed rank teste implemented in PAUP* 4.062a (Swofford, 2000), maximum-likelihood scores of the topologies were evaluated by the non-parametric Shimodaira-Hasegawa test (Shimodaira & Hasegawa, 1999) as implemented in PAUP* (Swofford, 2000). Finally, to test whether or not our data support monophyly of the genera in the Tropheini, we performed the topology-dependent permutation tail probability (T-PTP) test available in PAUP* (Swofford, 2000), using a constrained topology in which we forced monophyly of the genera, and 100 replications.

In the first step of the analysis the presence of a phylogenetic signal in the entire data set was tested by means of a likelihood mapping analysis using the computer program Puzzle 4.0 (Strimmer & von Haeseler, 1996). Likelihood mapping is a permutation method evaluating the likelihood support for the three alternative topologies of multiple sets of four taxa (quartets) to assess the overall phylogenetic signal in the data set. The likelihoods of the three alternative topologies of all evaluated taxon quartets are mapped on a triangle representing a barycentric coordinate system. The triangle is partitioned into seven distinct regions: the central region representing completely unresolved topologies, the three corners representing fully resolved topologies, and three intermediate regions between the corners representing partially resolved topologies. This analysis was carried out twice, with and without the outgroup taxa. Next, the relative rates of base substitutions were evaluated for all 42 ingroup taxa. We also carried out this test with a more inclusive data set representing all twelve Tanganyikan lineages. These tests were necessary to justify the use of genetic distances for estimating relative ages of the lineages of the Tropheini, as well as for the determination of relative ages with respect to other Tanganyikan lineages. To this end, we performed the branch length test, which is implemented in the computer program LINTRE (Takezaki et al., 1995).

For phylogenetic reconstruction we performed maximum parsimony, neighbor-joining and maximum likelihood analyses in parallel using the computer-program PAUP* 4.0b2a (Swofford, 2000). All base substitutions and indels were weighted equally in maximum parsimony, Kimura-2-Parameter (Kimura, 1980) and Jukes-Cantor distances (Jukes & Cantor, 1969) were used for neighbor-joining. The appropriate model of molecular evolution for the maximum likelihood analysis was evaluated by a likelihood ratio test implemented in the computer program Modeltest 3.0 (Posada & Crandall, 1998). This test justified the use of the HKY model of molecular evolution (Hasegawa et al., 1985) including invariable sites and a gamma correction. We applied the proportion of invariable sites (0.497), the gamma shape parameter (0.607), the base frequencies (A: 0.295; C: 0.259; G: 0.130; T: 0.316), and the transition/transversion ratio (4.407) estimated during the search. We assessed the robustness of the resulting topologies by applying standard measures of confidence (bootstrap and quartet-puzzling

Table 1. Characterization of the studied species

Tribe[1]	Taxon	GenBank Accession Number		Voucher		
		Cytochrome *b*	Control region	Specimen number[2]	Habitat	Diet
Haplochromini	*Astatotilapia burtoni*	Z21773[3]	Z215751[3]		swamps	Invertebrates
	Haplochromis obesus	Meyer et al., 1990[3]	U12552[3]		L. Victoria	
	Ctenochromis horei	AY301926	AY301952	1145-T1992	ubiquitous	Invertebrates
	Ctenochromis horei	AY301927	AY301953	1146-T1992	ubiquitous	Invertebrates
Limnochromini	*Gnathochromis pfefferi*	AY301928	AY301954	893-T1992	rock/cobble	Invertebrates
	Gnathochromis pfefferi	AY301929	AY301955	1089-T1992	rock/cobble	Invertebrates
Tropheini	*Limnotilapia dardennii*	AY301930	AY301956	244-T1992	rock/cobble	Invertebrates
	Limnotilapia dardennii	AY301931	AY301957	1158-T1992	rock/cobble	Invertebrates
	Lobochilotes labiatus	AY301932	AY301958	1376-T1992	rock	Invertebrates
	Lobochilotes labiatus	AF428170[3]	AF400733[3]		rock	Invertebrates
	Petrochromis ephippium	AY301933	AY301959	1084-T1992	rock	Aufwuchs
	Petrochromis famula	AY301934	AY301960	450-T1992	rock	Aufwuchs
	Petrochromis famula	AY301935	AY301961	579-T1992	rock	Aufwuchs
	Petrochromis famula	AY301936	AY301962	995-T1992	rock	Aufwuchs
	Petrochromis famula	AY301937	AY301963	1019-T1992	rock	Aufwuchs
	Petrochromis orthognathus	AY301938	AY301964		rock	Aufwuchs
	Petrochromis orthognathus	AY301939	AY301965		rock	Aufwuchs
	Petrochromis orthognathus	AY301940	AY301966	1242-T1992	rock	Aufwuchs
	Petrochromis polyodon "elongate"	AY301941	AY301967	391-T1992	rock	Aufwuchs
	Petrochromis polyodon "elongate"	AY301942	AY301968	603-T1992	rock	Aufwuchs
	Petrochromis polyodon "blue-fin"	AY301943	AY301969	692-T1992	rock	Aufwuchs
	Petrochromis polyodon "blue-fin"	AY301944	AY301970	693-T1992	rock	Aufwuchs
	Petrochromis sp. UBWARI	AY301945	AY301971		rock	Aufwuchs
	Petrochromis trewavasae	AY301946	AY301972	331-T1992	rock	Aufwuchs
	Pseudosimochromis curvifrons	AY301947	AY301973	1345-T1992	rock/sand	Aufwuchs
	Pseudosimochromis curvifrons	AY301948	AY301974	1346-T1992	rock/sand	Aufwuchs
	Simochromis babaulti	Z12045[3]	AF400736[3]		rock	Aufwuchs
	Simochromis babaulti	AY301949	AY301975	724-T1992	rock	Aufwuchs
	Simochromis babaulti	AY301950	AY301976	726-T1992	rock	Aufwuchs
	Simochromis diagramma	AY301951	AY301977	793-T1992	rock	Aufwuchs
	Tropheus brichardi NYANZA	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus duboisi	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus duboisi	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii BULU	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii CHAITIKA	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii IKOLA	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii KALA	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii KAVALLA	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii KIRIZA	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii LUPOTA	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii RUTUNGA	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii WAPEMBE	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus moorii ZONGWE	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs
	Tropheus polli BULU	Sturmbauer & Meyer, 1992[3]	Sturmbauer & Meyer, 1992[3]		rock	Aufwuchs

[1] According to Poll (1986)

[2] Deposited at the Royal Museum for Central Africa, Tervuren, Belgium

[3] Sequences published elsewhere

frequencies) with 1000 pseudo-replicates (bootstrap) and the evaluation of 10 000 random quartets (quartet-puzzling), and in the case of maximum likelihood under the fast stepwise addition option in PAUP∗ 4.0b2a (Swofford, 2000).

To evaluate the supports for distinct internal branches critical for the interpretation of the evolutionary pathways, a series of four-cluster likelihood analyses (Strimmer & von Haeseler, 1997) were performed. In this method four clusters of taxa are predefined to calculate the relative frequencies of each of the three possible topologies interrelating the members of the four taxon clusters. Likelihoods of all evaluated quartet trees for each subset of four taxa are mapped on a triangle. The triangle is now partitioned in three regions, each defining the likelihood frequencies of one of the three alternative topologies. This method also allows to delineating the onset of major diversification events, which are indicated by clusters of very short internal branches. For this analysis we used a reduced data-set of 17 taxa (plus one outgroup taxon) with a similar rate of base substitution according to the branch length test implemented in LINTRE (Takezaki et al., 1995). In the second data-set with a reduced number of taxa, each species or closely related assemblage, that was consistently identified by the first phylogenetic analyses, was represented by a single sequence only. From each cluster we selected the sequence with a root-to-tip distance that was closest to the average root-to-tip distance of 0.067 identified in the branch length test in LINTRE. For the second data-set, we again performed parsimony, neighbor-joining (Saitou & Nei, 1987) and maximum likelihood analyses and declaring *Astatotilapia burtoni* as outgroup. Modeltest 3.0 (Posada & Crandall, 1998) identified the HKY model (Hasegawa et al., 1985) with invariable sites and a gamma correction as optimal model of molecular evolution. We used the proportion of invariable sites (0.529), the gamma shape parameter (0.598), the base frequencies (A: 0.283; C: 0.264; G: 0.144; T: 0.309), and the transition/transversion ratio (4.566) estimated during the search.

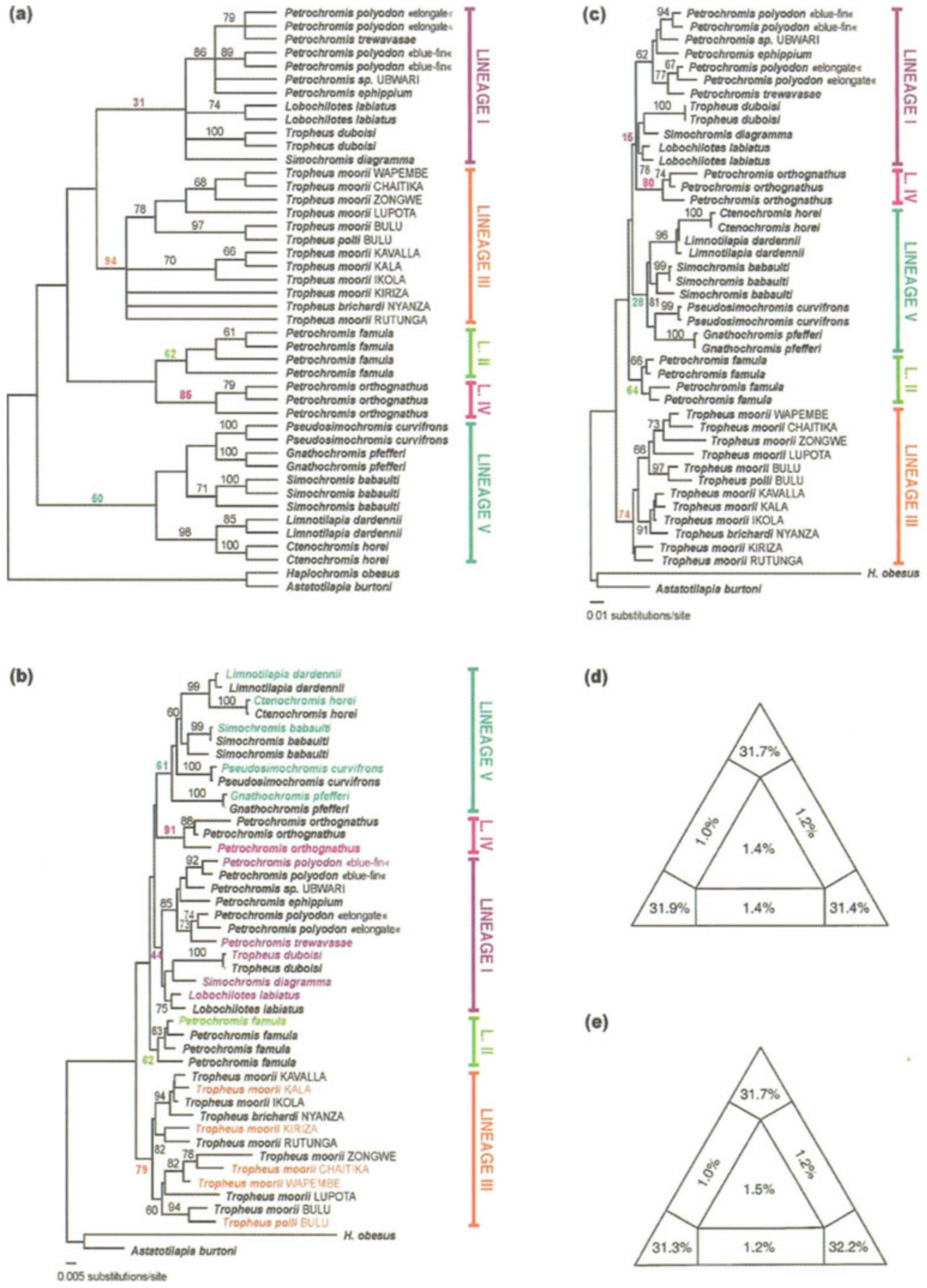
In the first three four-cluster analyses of the second data set the outgroup was defined as the first cluster and the five lineages identified were grouped into the remaining three clusters according to the different topologies found by the three tree-building methods. Then the outgroup was omitted and four new clusters were defined, depending on which discrete internal branch was under evaluation. The five lineages (defined according to the phylogenetic analyses) were clustered into the ten possible sets of quartets, and all 30 possible quartet topologies were tested for their relative supports.

As a complementary approach to estimate the relative age of the Tropheini and the time frame of lineage formation, we calculated the average genetic distances among all five lineages of the Tropheini by including all possible pairwise groupings of taxa. We then compared minimum, maximum and average Kimura distances of the Tropheini with those published for the Lamprologini and the genus *Tropheus* (Sturmbauer & Meyer, 1993; Sturmbauer et al., 1994; see also Baric et al., 2003). This comparison was based on sequences of the control region only.

Results

Phylogenetic analyses suggested the formation of five distinct lineages at the very base of the radiation of the Tropheini (named lineage I – V in Figs 1 and 2), which were consistently recovered in all analyses. Lineage I consisted of *Lobochilotes labiatus*, *Simochromis diagramma, Tropheus duboisi*, and a clade including *Petrochromis trewavasae*, *P.* sp. UBWARI, *P. polyodon* 'elongate', *P. polyodon* 'blue-fin', and *P. ephippium*. *Petrochromis polyodon* 'elongate' consistently clustered with *P. trewavasae*, while *P. polyodon* 'blue-fin' was resolved in a second clade in neighbor-joining and maximum-likelihood, together with *P.* sp. UBWARI and *P. ephippium.* Lineage II contained the four representatives of *Petrochromis famula*. Lineage III combined the different local forms of *Tropheus moori*, as well as *T. polli* and *T. brichardi*. Lineage IV comprised three specimens of *Petrochromis orthognathus*. Finally, lineage V consisted of *Gnathochromis pfefferi*, *Pseudosimochromis curvifrons*, *Simochromis babaulti*, and a clade formed by *Limnotilapia dardennii* and *Ctenochromis horei*.

The monophyly of four lineages (II, III, IV and V in Fig. 1) was supported by high numbers of synapomorphs, as well as by adequate bootstrap- and likelihood values in all analyses. The fifth lineage (lineage I) was consistently found by all three phylogenetic methods, but was defined by relatively few synapomorphs. The most striking observation was that – despite the excellent overall phylogenetic signal in the data set (Fig. 1d,e) – there was substantial conflict in the branching order that defined the emergence of the major lineages at the base of the radiation. Maximum parsimony yielded 32 most parsimonious trees of a tree length of 570 evolutionary steps (consistency index excluding uninformative sites, 0.41; retention index, 0.67; the strict consensus topology is depicted in Fig. 1a). Lineage V occupied the most ancestral branch in all 32 most parsimonious trees, sister group to a clade comprised by lineages II and IV, and a clade formed by lineages I and III. In neighbor-joining, lineage III occupied the most ancestral position, sister group to a clade containing lineage II, lineage V, lineage IV, and lineage I (Fig. 1b). In the maximum likelihood tree lineage III formed the most ancestral branch, followed by a clade comprising lineages II, I, IV and V (Fig. 1c). Neither in the Wilcoxon signed rank test ($P < 0.05$) nor in the Shimodaira-Hasegawa test ($P < 0.05$) a particular topology was favored.


(a)
Petrochromis polyodon »elongate«
Petrochromis polyodon »elongate«
Petrochromis trewavasae
Petrochromis polyodon »blue-fin«
Petrochromis polyodon »blue-fin«
Petrochromis sp. UBWARI
Petrochromis ephippium
Lobochilotes labiatus
Lobochilotes labiatus
Tropheus duboisi
Tropheus duboisi
Simochromis diagramma
Tropheus moorii WAPEMBE
Tropheus moorii CHAITIKA
Tropheus moorii ZONGWE
Tropheus moorii LUPOTA
Tropheus moorii BULU
Tropheus polli BULU
Tropheus moorii KAVALLA
Tropheus moorii KALA
Tropheus moorii IKOLA
Tropheus moorii KIRIZA
Tropheus brichardi NYANZA
Tropheus moorii RUTUNGA
Petrochromis famula
Petrochromis famula
Petrochromis famula
Petrochromis famula
Petrochromis orthognathus
Petrochromis orthognathus
Petrochromis orthognathus
Pseudosimochromis curvifrons
Pseudosimochromis curvifrons
Gnathochromis pfefferi
Gnathochromis pfefferi
Simochromis babaulti
Simochromis babaulti
Simochromis babaulti
Limnotilapia dardennii
Limnotilapia dardennii
Ctenochromis horei
Ctenochromis horei
Haplochromis obesus
Astatotilapia burtoni
LINEAGE I
LINEAGE III
L. II
L. IV
LINEAGE V
79
86
89
31
74
100
68
78
97
94
70
66
61
62
85
79
100
100
100
71
60
85
98
100
(b)
Limnotilapia dardennii
Limnotilapia dardennii
Ctenochromis horei
Ctenochromis horei
Simochromis babaulti
Simochromis babaulti
Simochromis babaulti
Pseudosimochromis curvifrons
Pseudosimochromis curvifrons
Gnathochromis pfefferi
Gnathochromis pfefferi
Petrochromis orthognathus
Petrochromis orthognathus
Petrochromis orthognathus
Petrochromis polyodon »blue-fin«
Petrochromis polyodon »blue-fin«
Petrochromis sp. UBWARI
Petrochromis ephippium
Petrochromis polyodon »elongate«
Petrochromis polyodon »elongate«
Petrochromis trewavasae
Tropheus duboisi
Tropheus duboisi
Simochromis diagramma
Lobochilotes labiatus
Lobochilotes labiatus
Petrochromis famula
Petrochromis famula
Petrochromis famula
Petrochromis famula
Tropheus moorii KAVALLA
Tropheus moorii KALA
Tropheus moorii IKOLA
Tropheus brichardi NYANZA
Tropheus moorii KIRIZA
Tropheus moorii RUTUNGA
Tropheus moorii ZONGWE
Tropheus moorii CHAITIKA
Tropheus moorii WAPEMBE
Tropheus moorii LUPOTA
Tropheus moorii BULU
Tropheus polli BULU
H. obesus
Astatotilapia burtoni
0.005 substitutions/site
LINEAGE V
L. IV
LINEAGE I
L. II
LINEAGE III
99
100
60
99
61
100
100
88
91
92
85
74
73
100
44
75
63
62
94
82
82
78
79
60
94
(c)
Petrochromis polyodon »blue-fin«
Petrochromis polyodon »blue-fin«
Petrochromis sp. UBWARI
Petrochromis ephippium
Petrochromis polyodon »elongate«
Petrochromis polyodon »elongate«
Petrochromis trewavasae
Tropheus duboisi
Tropheus duboisi
Simochromis diagramma
Lobochilotes labiatus
Lobochilotes labiatus
Petrochromis orthognathus
Petrochromis orthognathus
Petrochromis orthognathus
Ctenochromis horei
Ctenochromis horei
Limnotilapia dardennii
Limnotilapia dardennii
Simochromis babaulti
Simochromis babaulti
Simochromis babaulti
Pseudosimochromis curvifrons
Pseudosimochromis curvifrons
Gnathochromis pfefferi
Gnathochromis pfefferi
Petrochromis famula
Petrochromis famula
Petrochromis famula
Petrochromis famula
Tropheus moorii WAPEMBE
Tropheus moorii CHAITIKA
Tropheus moorii ZONGWE
Tropheus moorii LUPOTA
Tropheus moorii BULU
Tropheus polli BULU
Tropheus moorii KAVALLA
Tropheus moorii KALA
Tropheus moorii IKOLA
Tropheus brichardi NYANZA
Tropheus moorii KIRIZA
Tropheus moorii RUTUNGA
H. obesus
Astatotilapia burtoni
0.01 substitutions/site
LINEAGE I
L. IV
LINEAGE V
L. II
LINEAGE III
94
62
67
77
100
16
78
74
80
100
96
99
28
81
99
100
66
64
73
66
97
74
91
(d)
31.7%
1.0%
1.2%
1.4%
31.9%
1.4%
31.4%
(e)
31.7%
1.0%
1.2%
1.5%
31.3%
1.2%
32.2%

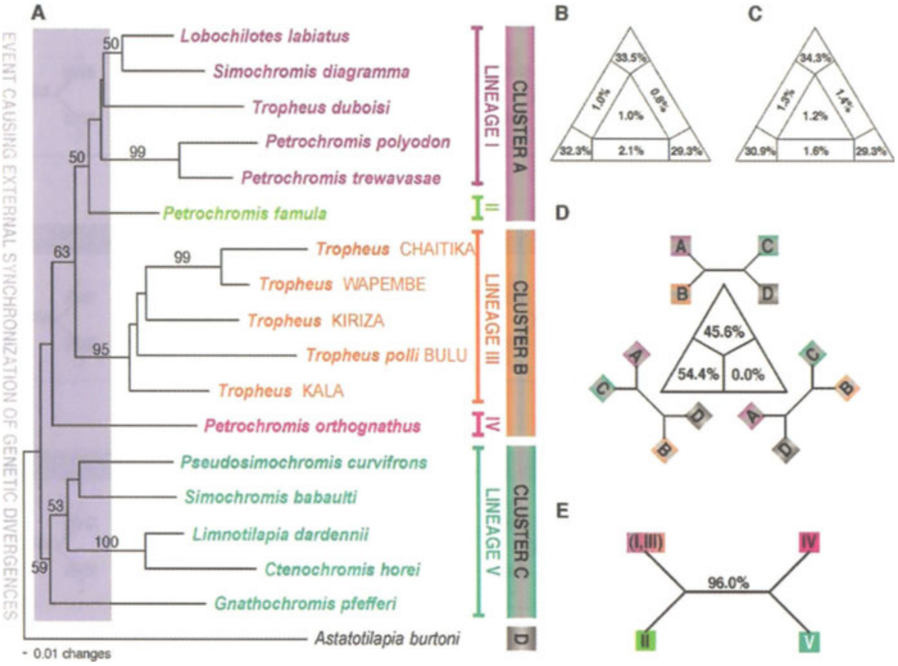


Figure 2. One example for a four-cluster likelihood analysis (Strimmer & Von Haeseler, 1997) evaluating the support for distinct internal branches. For this analysis a reduced data-set inlcuding 17 taxa (plus the outgroup *Astatotilapia burtoni*) was used. (a) Neighbor-joining tree based upon Kimura-2-parameter distances of a 402 bp segment of the cytochrome *b* and a 385 bp segment of the control region. (b), (c) Likelihood mapping analysis demonstrating the presence of a strong overall phylogenetic signal in the data set including (b) and excluding (c) the outgroup. (d) Four-cluster likelihood mapping analysis testing the relative likelihoods of the three alternative topologies of the four taxon-clusters (A, B, C and D in (a)) derived from the neighbor joining phylogeny. None of the three possible topologies is clearly favored pointing to a lack of phylogenetic signal in these basal branches. This suggests a contemporaneous origin of the lineages. Using this method we tested all alternative topologies that were obtained in our phylogenetic analyses using alternative tree building algorithms. (e) Example of a quartet topology excluding the outgroup with a different grouping of lineages in comparison to (d). See text and Figure 3 for further details.

The topology dependent T-PTP test, however, rejected monophyly of the genera ($P = 0.01$).

The maximum parsimony analysis of the second data set with a reduced number of taxa yielded three most parsimonious trees of a length of 305 steps (consistency index excluding uninformative sites, 0.48; retention index, 0.54, tree not shown). Lineage II was resolved as sister group to the remaining four lineages in all three most parsimonious trees. In the neighbor-joining tree, lineage V was resolved as sister group to the remaining four lineages, sister group to a clade containing lineage IV, lineage III, lineage II, and lineage I (Fig. 2a). In the maximum likelihood tree, lineage III formed the most ancestral branch, sister

⟵

Figure 1. Phylogenetic analyses of the endemic Tanganyikan tribe Tropheini based upon 402 bp of the mitochondrial cytochrome *b* gene and 385 bp of the mitochondrial control region. *Astatotilapia burtoni* and *Haplochromis obesus* were used as outgroups. Boostrap values larger than 60% are depicted above the corresponding branches, except for the branches defining the five major lineages (I, II, III, IV, V), for which the values were also depicted when they were smaller than 60%. The generally short internal branches at the basis highlight the rapidity of lineage formation in this group of cichlid fishes. (a) Strict consensus tree obtained from the 32 most parsimonious trees (tree length, 570; consistency index excluding uninformative sites, 0.41). (b) Neighbor-joining topology based upon Kimura-2-parameter distances. (c) Maximum likelihood tree applying the HKY+I+Γ model for molecular evolution. Further details are given in the material and method section. (d), (e) Likelihood mapping analysis (Strimmer & von Haeseler, 1997) demonstrating the presence of a strong overall phylogenetic signal in the data set including (d) and excluding (e) the outgroup.

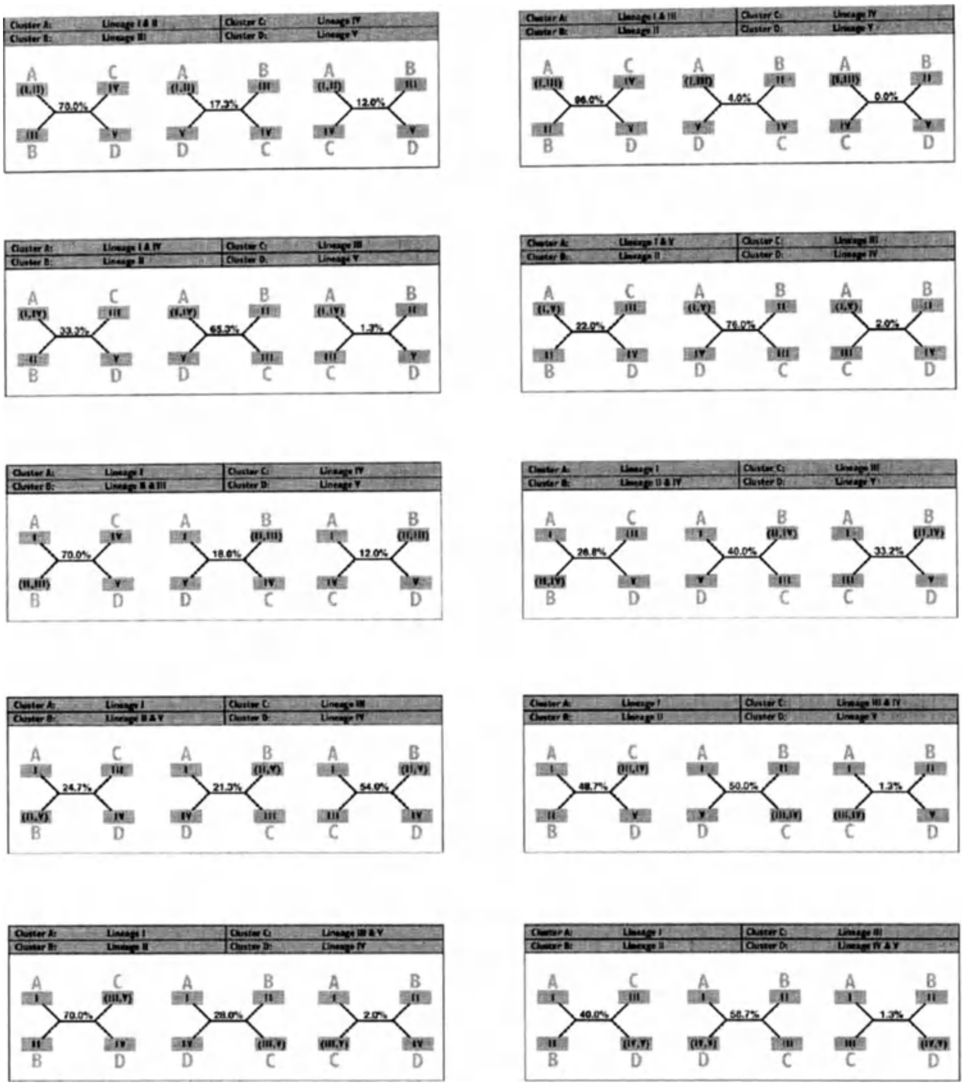


Figure 3. Four-cluster likelihood mapping analyses of 30 possible alternative topologies of the five lineages identified in the Tropheini (I–V). The lineages are defined in Figures 1 and 2. Each box contains the results of one four-cluster likelihood mapping analysis of 10 possible four cluster-groupings of the five lineages. The relative percentage fractions were inconsistent with respect to the tested topologies and highlight the lack of a clear phylogenetic signal.

group to lineage IV and a clade comprising lineages IV, V and I (not shown).

In the four-cluster likelihood mapping analyses, in which all alternative topologies from the three tree building algorithms of the reduced data-set were systematically evaluated, the absence of phylogenetic signal at the base of the radiation became further evident. None of the four-cluster likelihood mapping analyses clearly favored one particular topology. This is depicted for the neighbor-joining tree in Figure 2d, in which two out of the three possible cluster topologies were found to be equally favored (45.6%; 54.4%;

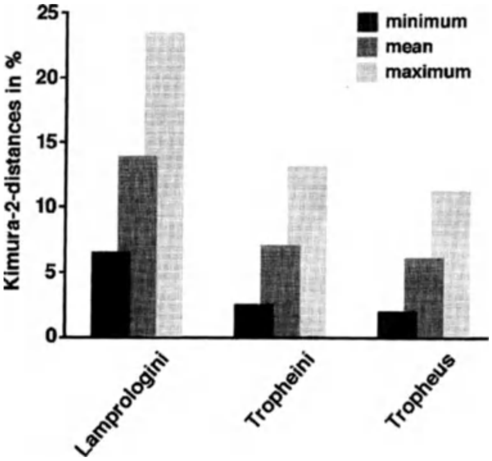


Figure 4. Minimum, average and maximum Kimura distances among lineages of the Tanganyikan tribes Lamprologini (seven lineages, Sturmbauer et al., 1994) and Tropheini (five lineages), compared to those found within the genus *Tropheus* alone (Sturmbauer & Meyer, 1992; Sturmbauer et al., 1997; Baric et al., 2003), based upon 383 bp of the control region. The comparison highlights the younger evolutionary age of the Tropheini as compared to the Lamprologini. The Lamprologini must have undergone radiation during an earlier phase in the lake history. The almost identical ranges of genetic distances observed in the Tropheini and the genus *Tropheus* alone indicates an immediate subdivision of the *Tropheus*-lineage (Lineage III in Fig. 1a) after its formation.

0.0%). The same was found in the four-cluster likelihood analyses of the maximum likelihood tree (46.7%; 50.0%; 3.3%), and of the three most parsimonious trees (59.0%; 34.7%; 6.3%). The lack of support for one particular branching order of the five lineages was further signaled by the fact that the most highly supported topologies differed in all three algorithms. The four-cluster likelihood mapping analyses of all 30 possible groupings of the five lineages also gave conflicting results and did not favor one particular branching order (Fig. 3).

The comparison of genetic distances corroborates the almost contemporaneous origin of the five lineages. The average genetic distances among the five lineages of the Tropheini amounted to 7.01% (standard deviation = 0.018; minimum = 2.57%; maximum = 13.28%; number of pairwise comparisons = 665). Moreover, the average genetic distance within the genus *Tropheus* alone (lineage III) was only slightly smaller than that observed among the five lineages of the Tropheini (average = 6.14%; minimum = 4.6; maximum 7.9%; see Baric et al., 2003). This observation suggests that *Tropheus* diversified further immediately after its emergence (Fig. 4). The smaller average genetic distances among the five lineages of the Tropheini in relation to those observed among lineages of the Lamprologini (Sturmbauer et al., 1994) indicate that the Tropheini must thus have undergone radiation at a much later stage than the Lamprologini. According to a tentative calibration of the control region of East African cichlid fishes a Kimura-distance of 7.01% would translate into an age between 807 ka and 1092 ka (Sturmbauer et al., 2001).

Discussion

A stunningly rapid pace of speciation and cladogenesis, particularly at early stages of adaptive radiation, has been repeatedly demonstrated for African cichlid fishes (Meyer et al., 1990; Sturmbauer & Meyer, 1992, 1993; Sturmbauer et al., 1994; Verheyen et al., 1996, 2003; Rüber et al., 1998, 1999; Nagl et al., 1998, 2000; Salzburger et al., 2002b). Despite their rapid pace, speciation events in cichlid fishes were assumed to proceed in a bifurcating and tree-like fashion (Coulter, 1994; Ribbink, 1994; Turner, 1994). Bush phylogenies may be either interpreted as ‘soft polytomy’ – an analytical artefact, e. g., due to a lack of resolution of the gene segment analyzed and the phylogenetic method used – , or a ‘hard polytomy’ – generated by a truly simultaneous branching (Maddison, 1989, see Jackman et al., 1999 for discussion). Our phylogenetic analysis suggests a truly bush-like manner of cladogenesis in the Tropheini. The contemporaneous formation of five major lineages is independently supported by three findings. First, the internal branches interrelating these lineages were extremely short leading to a substantial degree of conflict in the resulting tree topologies, despite the excellent overall phylogenetic signal in the data set. Second, these alternative topologies turned out to be equally supported in the four-cluster lieklihood mapping analyses, the Wilcoxon signed rank test and the Shimodaira-Hasegawa test. Third, the relative phylogenetic distances among the lineages were strikingly similar. Given the strength of the overall phylogenetic signal in the data set, such a lack of phylogenetic signal in the ancestral branches can only be interpreted as the consequence of a rapid cladogenesis event.

This leads us to suggest that the observed pattern of evolution is the consequence of a contemporaneous origin of several lineages of the Tropheini, and not an

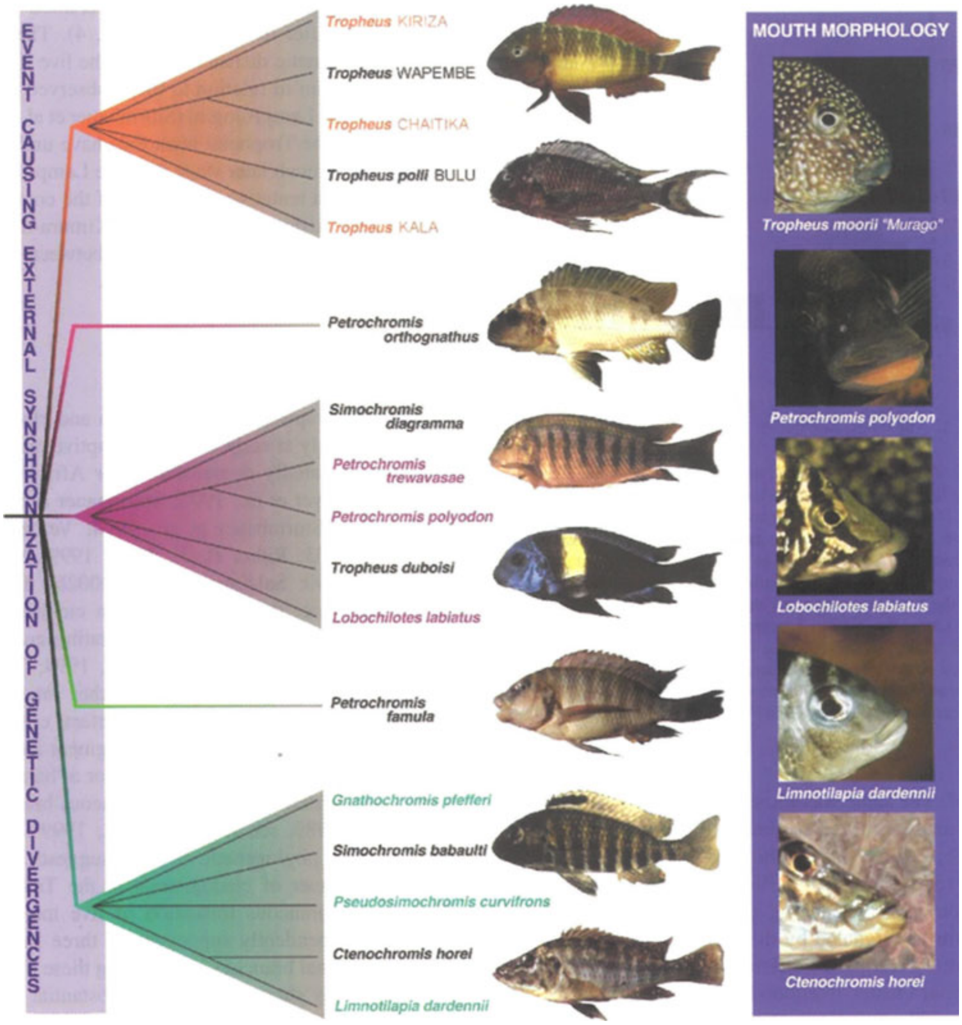


Figure 5. Phylogenetic hypothesis for the radiation of the Tropheini based upon a strict consensus tree of parsimony, neighbor-joining and maximum likelihood, indicating that five distinct lineages arose contemporaneously, triggered by an environmental change. The depicted species (names are in black) and mouth morphologies underline the extreme degree of eco-morphological diversity within and among the five lineages. The paraphyletic placement of members of the genera *Tropheus*, *Simochromis* and *Petrochromis* further points to a high probability of recurrent evolution of corresponding morphologies under this mode of speciation, due to the action of similar selective forces in geographically isolated habitats of the same type.

artifact resulting from the limited power of resolution of the phylogenetic analyses. Concerted cladogenesis can only be induced by a synchronized vicariance event, triggered by external influences enforcing dramatic habitat changes. According to various models of allopatric speciation temporary spatial isolation of populations is caused by the discontinuous distribution of suitable habitats in an ecosystem, and the influence of external events changing habitats and/or their distribution (Sturmbauer, 1998). In the case of African cichlid fishes, lake level fluctuations were shown to be powerful modulators of habitat distribution (Sturmbauer, 1998; Sturmbauer et al., 2001). Taking into account that Lake Malawi rose by about 100 m within the last 200 years (Owen et al., 1990), one of the most striking characteristics of lake level fluctuations is their rapidity. The onset of spatial and hence genetic isolation is thus effectively synchronized by a rapid rise of the lake level, enforcing an almost concurrent split of lowstand populations into several units. Two recent studies showed that such synchronous population subdivisions are clearly mirrored in the observed patterns of genetic relatedness of cichlid populations in Lakes Victoria, Malawi and Tanganyika (Sturmbauer et al., 2001; Baric et al., 2003). Populations arising from the same vicariance event show equal genetic distances to each other. In relation to the pace of molecular evolution of mitochondrial genes, a bush-phylogeny interrelating all lineages arising from such a synchronized dispersal incident is the inevitable consequence (Fig. 5). It may also be noted here that the degree of genetic variation of all lineages arising from the same vicariance event depends on the genetic diversity of the founder population at the time of subdivision. This diversity defined the starting point of lineage sorting during the process of coalescence.

An equal age of lineages does not imply that subsequent speciation events in each of the founder populations occurred concurrently. Speciation events may happen at different times in any of those populations, since they depend on local selective forces. Extant species belonging to different lineages that were once formed contemporaneously are almost equidistant in terms of genetic distances to each other, while species originating in the same lineage may show smaller and different genetic distances to each other. When several populations are formed at the same time – e.g. by a major rise of the lake level –, several lineages may subsequently undergo speciation. Explosive speciation via synchronization of genetic divergence seems to be more likely at advanced stages of adaptive radiation, since the involved species must have particular biological characteristics providing the potential to undergo further diversification. Their ability for active dispersal over ecological barriers must be limited, so that major dispersal events can only be induced externally by habitat shifts such as lake level fluctuations. It should be noted that in some cases populations may also be split by a decrease of the lake level, for example when islands become exposed.

A series of molecular phylogenetic analyses suggested *Astatotilapia burtoni* as the closest relative of the Tropheini. This species is widely distributed in swampy areas and rivers in and around Lake Tanganyika and does not seem to have a reduced ability for dispersal at all. Our phylogeny suggests that the evolution of a reduced ability for active dispersal due to specialization to patchy niches may represent a key innovation allowing to undergoing speciation via synchronization of genetic divergence. This feature must have evolved at the very beginning of the radiation in the common ancestor of the Tropheini. The ancestral species has most likely gone extinct due to competition of better adapted descendants (see Sturmbauer, 1998 p. 23).

Our phylogenetic analyses further confirmed *Gnathochromis pfefferi* as a member of the Tropheini (Kocher et al., 1995, Salzburger et al., 2002b) and also suggested the placement of the haplochromine cichlid *Ctenochromis horei* in this tribe (see also Nishida, 1997). Another striking observation from our phylogeny – corroborating previous results based on allozyme data (Nishida, 1997) – is its frequent conflict with the present taxonomic assignments suggesting that several genera may be paraphyletic, so that corresponding trophic specializations may have evolved repeatedly within a single lake. This was observed for the genus *Tropheus* in which *T. duboisi* was consistently placed outside the lineage comprising its congeneric allies, as well as for the genera *Petrochromis* and *Simochromis* (Fig. 5). All these genera were defined primarily on the basis of their particular trophic morphology (Boulenger, 1898; Yamaoka, 1983; Poll, 1986). Such a high frequency of recurrent evolution of convergent morphologies can best be explained as an inherent feature of the proposed mode of speciation, since geographically isolated habitats of the same type should create similar selective forces on their species communities. Moreover, natural selection acted on the same set of colonizing species, so that the initial conditions were likely to be similar in all founder populations. Particular ecological and morphological adaptations, which provide effective resource partitioning, may have been independently favored by natural selection in isolated communities. Under these particular environmental conditions, Stephen J. Gould's mind experiment of 'replaying life's tape' (Gould, 1990) has been performed by nature to indeed produce similar evolutionary outcomes. As a consequence, morphology-based taxonomic analyses interpreting similar pharyngeal morphologies as synapomorphs, justifying the placement of eco-morphologically equivalent taxa in a single genus, may often be mislead.

Ancient incomplete lineage sorting was recently suggested as explanation for the para- or polyphyletic placement of inserted retroposons (SINE) among lineages of cichlid fishes in Lake Tanganyika (Takahashi et al., 2001). Incongruence of genetic characters can have two causes: homoplasy and ancestral polymorphism. O'hUigin et al. (2002) argued in a model study on anthropoid primates that homoplasy is a more likely contributor to incongruence in more ancient cladogenesis events, while the persistence of ancestral polymorphism is more likely in younger evolutionary splits. When ancestral polymorphisms undergo lineage sorting, a gene phylogeny that is incongruent to the species phylogeny might be the result. After the divergence of each lineage, alternative nuclear alleles or mitochondrial haplotypes might become fixed stochastically. It is important to note that it is the relative number of incongruent character state changes, which might mislead phylogenetic analyses. Mitochondrial genes tend to evolve more rapidly than nuclear genes and lineage sorting occurs faster due to maternal inheritance, so that the time window of phylogenetic resolution is shorter than for nuclear loci. Our analysis of the Tropheini points to a great degree of conflict. This conflict, however, is mainly due to the lack of phylogenetically informative characters in the ancestral branches. Ancient incomplete lineage sorting, however, might also contribute to the observed conflicts in the phylogenetic analysis of the Tropheini, and it should thus be seen as another indicator for extremely rapid cladogenesis.

All morphologically distinguishable taxa were also genetically distinct in our mitochondrial phylogeny. This observation is in clear contrast to analyses on Lake Malawi and Victoria cichlids (Moran & Kornfield, 1993; Parker & Kornfield, 1997; Nagl et al., 1998). However, this is congruent to the age estimate for the genus *Tropheus*, which may be five token times the age of the entire Lake Victoria cichlid species flock and about the same age as the Lake Malawi cichlid flock (Sturmbauer & Meyer, 1992; Verheyen et al., 2003).

The scenario of speciation proposed here does not rule out the occurrence of sympatric speciation within any geographically isolated population. Species arising from sympatric or micro-allopatric speciation events are more closely related to each other, but they exhibit the same genetic distances to any species of a different population that arose from the primary vicariance event. Previous works on sticklebacks reported a similar evolutionary scenario, in which species gradually colonized newly emerging lakes during glacial retreat and repeatedly evolved eco-morphologically equivalent pairs of sympatric species (Schluter & McPhail, 1992; Taylor & McPhail, 2000). The crucial difference to the scenario proposed here is the relatively slow succession of repeated colonization events and their origin from more than one refugee population. In the Tropheini, recurrent evolution of equivalent species pairs is not connected to repeated colonization events.

Our data suggest that speciation via synchronization of genetic divergence is likely to be an important promoter of species multiplication in flocks of African cichlid fishes. We are convinced that this pattern of speciation will also be found in other organisms, whenever the ancestral species are already adapted to discontinuous habitats, which rapidly shift due to externally induced events.

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Remote sensing of vegetation and flooding on Magela Creek Floodplain (Northern Territory, Australia) with the SIR-C synthetic aperture radar

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Abstract

The potential of using synthetic aperture radar (SAR) imagery to study seasonal variations in floodplain inundation and accompanying changes in herbaceous plant communities was assessed for aquatic environments in the Kakadu region of northern Australia. Dual wavelength (C- and L-band), co- and cross-polarized (HH and HV) SAR imagery of the floodplain of Magela Creek, a tributary of the East Alligator River, was acquired in April and October 1994 during the Spaceborne Imaging Radar-C (SIR-C) missions of the Space Shuttle. The results indicate the effectiveness of multifrequency, polarimetric SAR as follows: woody and herbaceous vegetation in flooded and nonflooded states can be mapped. Aquatic, herbaceous macrophyte communities, such as *Nelumbo nucifera*, *Pseudoraphis* grassland and *Hymenachne-Eleocharis* swamp, can be distinguished. Phenologic changes in macrophyte communities between April and October are apparent. The similarity of the Magela floodplain to the extensive floodplains in northern Australia suggests that these results can be extended regionally.

Introduction

The Kakadu region of Australia's Northern Territory supports extensive wetlands including mangroves, seasonally inundated freshwater floodplains, salt flats, and small permanent lakes. Kakadu National Park, constituting about 70% of the region, has been listed under both the Ramsar Convention on Wetlands of International Importance and the UNESCO World Heritage Convention, in recognition of the outstanding diversity of its wetland habitats and its importance to large numbers of waterfowl. Seasonally inundated floodplains, which annually experience extremes of flood and drought, cover about 195 000 ha along the lower reaches of the region's major rivers (West Alligator, South Alligator, and East Alligator) and creeks (Magela and Cooper) (Finlayson & Woodroffe, 1996).

Duration of the inundation period is a major determinant of the species composition, areal extent, and phenology of floodplain plant communities (Finlayson et al., 1989). The mixture of shallow open water, emergent aquatic vegetation, and fringing grasslands that occurs during the dry-down period provides a critical habitat that attracts waterbirds from areas well beyond the region (Morton et al., 1993). Knowledge of flooding extent and duration is therefore an essential element in management of the Kakadu wetlands and of large areas of similar subcoastal wetlands of the Northern Territory. Such monitoring is feasible only using remote sensing.

Synthetic aperture radar (SAR), with its unique ability to detect flooding beneath vegetation canopies and to penetrate cloud cover, is well suited to the study of tropical wetlands. The wavelengths used by SAR sensors are sensitive to the size, density, and orientation of canopy elements such as leaves and small branches at C-band, and larger branches and tree trunks at L-band; SAR sensors have successfully been used in a variety of ecological applications involving vegetation structure (Kasischke et al., 1997). While flooding beneath a closed forest canopy cannot be detected with optical sensors such as Landsat Thematic Mapper, L-band SARs can penetrate even dense tree canopies. Double-bounce scattering between water surfaces and tree trunks or branches increases the strength of the signal backscattered to the sensor, making it possible to reliably detect inundation (Richards et al., 1987; Smith, 1997).

The floodplain and surrounding lowland vegetation of the Kakadu region differ in significant respects from those found in other tropical and subtropical sites where use of SAR has been demonstrated for wetland applications (Hess et al., 1995; Pope et al., 1997; Townsend, 2001). Richards et al. (1987) described enhanced LHH returns from seasonally inundated *Eucalyptus camaldulensis* forests in New South Wales and Victoria, and Imhoff et al. (1997) related multi-frequency backscattering from *Melaleuca* woodland sites near the South Alligator River to a variety of stand structural measures; however, SAR-based studies of Australian macrophyte communities have not been previously been reported.

The dominant vegetation of the lowlands bordering the Kakadu region's subcoastal floodplains is eucalypt open forest, woodland, and open-woodland with tall grass understory (Wilson et al., 1996). The *Melaleuca* open forest and woodland of the floodplains range widely in stand density. Some of Kakadu's perennial macrophyte communities are dominated by unusually large herbaceous species such as *Nelumbo nucifera*, which has leaves 40–50 cm wide on stalks up to 2 m tall (Sainty & Jacobs, 1994). The co-occurrence of low-biomass woodlands and macrophytes with large stalks suggests that flooded macrophyte, flooded woodland, and nonflooded woodland could be difficult to distinguish on SAR imagery. Dual-wavelength, co- and cross-polarized SAR imagery of Magela Creek, a tributary of the East Alligator River, was acquired from the Space Shuttle in April and October 1994 during the Spaceborne Imaging Radar-C (SIR-C) missions (Stofan et al., 1995). The study described here used the SIR-C datasets to test the potential of SAR to map freshwater wetland communities in subcoastal Northern Territory, and to monitor seasonal changes in floodplain inundation and in the extent and structure of herbaceous plant communities.

Study area

Magela Creek has a catchment of about 605 km^2. The creek descends from the dissected sandstone escarpment of the Arnhem Land Plateau to an erosional plain, flowing in braided sand channels until the floodplain widens in the Mudginberri Corridor (Fig. 1) and the watercourse becomes a series of billabongs and connecting channels. It then extends onto a seasonally inundated black-clay floodplain with permanent billabongs before discharging into the East Alligator River (Finlayson et al., 1989). The climate is classified as summer rainfall-tropical. Ninety-two percent of the mean annual rainfall of 1460 mm falls in intense storms during the hot and humid wet season, from November to March; warm, dry conditions prevail in the April–October dry season (McQuade et al., 1996). Floodplain inundation begins around January, resulting mainly from creek and overland flow rather than from direct precipitation. Water depths on the floodplain reach up to several meters during peak high water in March, then slowly decline with the end of the wet season. By the end of the dry season, standing water on the floodplain is restricted to isolated billabongs and swamps.

Magela Creek and its floodplain have been much studied, due to the presence of a uranium mine in the creek's catchment and the resulting need to understand the fate of potential pollutants in the event of a waste water release from the mine. Finlayson et al. (1989) mapped 10 major floodplain plant communities using aerial photographs, categorized macrophyte species occurring on the floodplain by growth strategy, growth form, and broad habitat, and measured the spatial and temporal variability of macrophyte communities along a transect at four stages from the late-wet to late-dry seasons. Forty percent of the floodplain was mapped as open forest, woodland, or open woodland dominated by one or more species of *Melaleuca*, and the remaining area as grassland, sedgeland, herbland, or mixtures thereof. Dominant herbaceous species include *Oryza meriodionalis, Hymenachne acutigluma, Eleocharis* spp., *Pseudoraphis spinescens, Nelumbo nucifera,* and *Nymphoides indica.* The pattern of vegetation variation is a function of both the flooding and drying phases, and duration of the inundation period is a major determinant of floodplain vegetation composition. Plant growth strategy is related to inundation period also: about 60% of species occurring in seasonally inundated areas are annuals, while permanently flooded areas have less than 40% annual species.

Because the timing of the two SIR-C data acquisitions corresponded to the late-wet and late-dry seasons, it is possible to compare the floodplain in a condition where it was completely inundated, with macrophytes near peak biomass, to one where floodwaters had receded and macrophytes were largely senescent. Lowland conditions also can be expected to vary between the two dates: surface soils dry well below wilting point during the dry season, and the

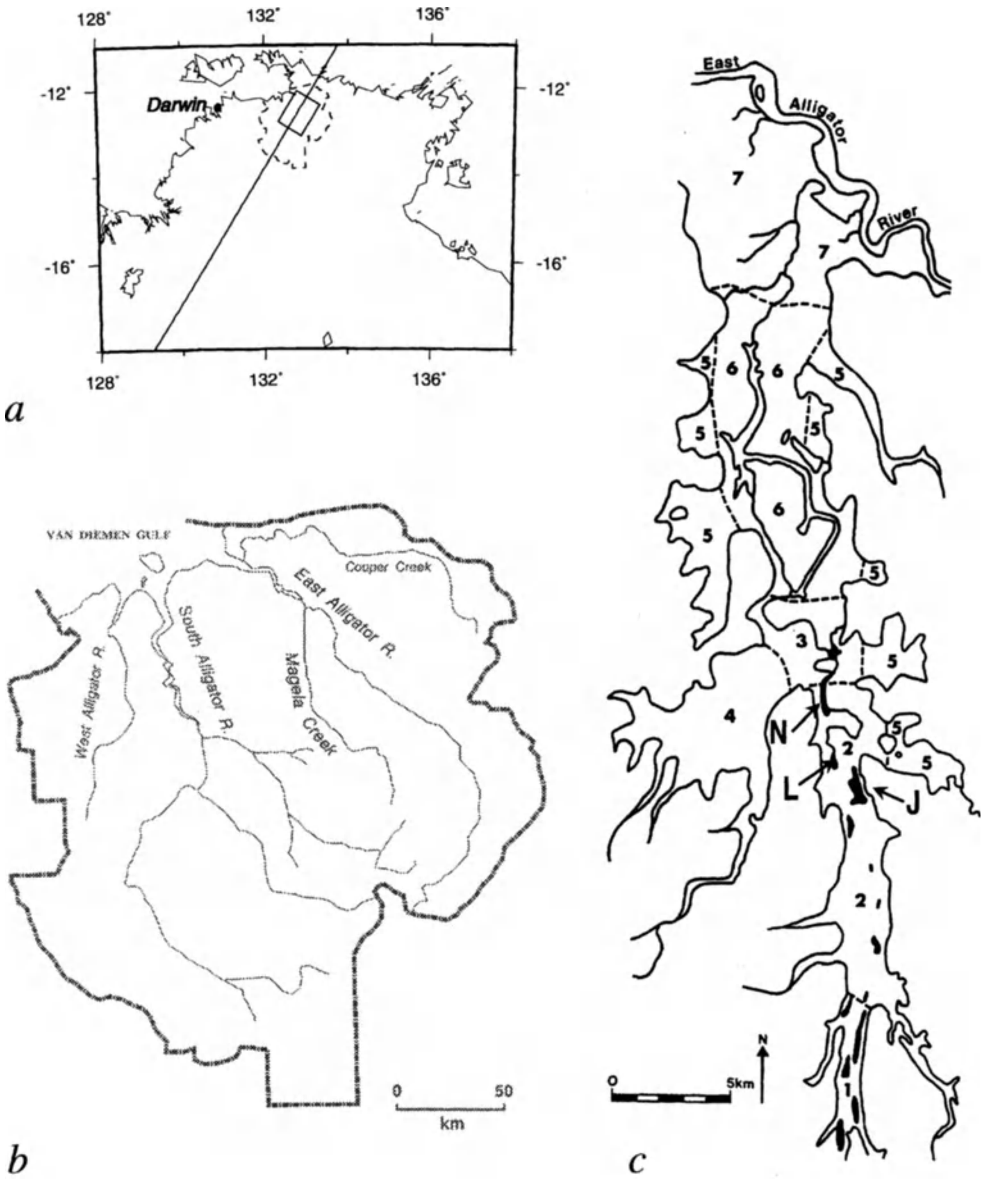

Figure 1. Location maps showing (a) SIR-C track, Alligator River scene, and Kakadu region; (b) rivers and creeks of Kakadu region; (c) major topographic divisions of Magela floodplain, following Hart & McGregor (1982): (1) Mudginberri corridor; (2) upper basin, (3) central topographic high, (4) Magela Plain, (5) back-plain swamps, (6) lower basin, (7) East Alligator floodplain. Letters with arrows indicate Jabiluka (J), Leichhardt (L), and Nankeen (N) billabongs.

grassy understory of the eucalypt woodlands senesces. Fire is a frequent, often annual occurrence during the late dry season, burning dry floodplain areas as well (Andersen, 1996).

Methods

Field verification and ancillary information

In order to verify ground conditions during the two SIR-C acquisitions, helicopter-based, oblique video surveys were carried out on 20 April 1994 and 18 October 1994. Both surveys followed the same flight plan, a series of 18 GPS-guided segments flown at an altitude of about 600 m, traversing areas of interest on the floodplain. Devonport et al. (1994) and Waggitt et al. (1995) provide detailed descriptions of the surveys, including waypoints and flight logs noting features such as vegetation type, presence or absence of flooding, and fire scars. Additional commentary on the videotapes was provided by C. M. Finlayson.

The Finlayson et al. (1989) vegetation map of the Magela floodplain was used to guide image interpretation. Due to the dynamic nature of the herbaceous plant cover on the floodplain, plant communities must be defined temporally as well as spatially; the mapping done by Finlayson et al. (1989) was specific to the high-water, maximum biomass period, approximately March to May, and was based on aerial photos and field transects acquired between 1983 and 1986. Because the April SIR-C acquisition coincided with the high-water, high-biomass period, the map was used to guide analysis of the SAR data. However, caveats given by Finlayson et al. (1989) limit the extent to which the map can be used to interpret imagery obtained a decade after the map was prepared. Yearly variations in rainfall and flooding affect the short-term composition of floodplain plant communities. Significant shifts in species dominance occur between years, and the spatial distribution of communities changes along environmental gradients, particularly water depth. A decline since the mid-1980s in the populations of feral water buffalo, which previously impacted vegetation both directly and through affecting surface hydrology, may have further altered plant communities. Because of this variability, the map was not suitable for training or testing image classification algorithms; however, it was used to create a digital mask of the floodplain boundary, to compare the mapped distribution of relatively static communities such as *Melaleuca* forest/woodland with SAR results, and to note herbaceous communities having spatial distributions that corresponded with definable patches in the SIR-C data.

Image analysis

The Alligator River segments of SIR-C data swaths 117.6 (April 1994) and 117.52 (October 1994) were used in the analysis (Fig. 2). The data are similar in terms of incident angle and imaging mode (SIR-C mode 11, HH and HV polarizations at C- and L-bands). However, because the April scene was acquired in lower resolution mode, its pixel dimensions are twice as large (Table 1). Image analysis emphasized the Magela Creek floodplain. A 27.5 × 38.75 km subscene centered on Magela floodplain was extracted from the Alligator River segment of the data. The April Magela subscene was super-sampled by a factor of two to the same pixel size as the October scene to facilitate coregistration. The April and October subscenes were then coregistered by a simple coordinate translation. The vegetation map was scanned and coregistered to the Magela subscenes using a second-order polynomial warp.

After pulses transmitted by a SAR sensor are reflected, scattered, and/or absorbed at the earth's surface, the intensity and timing of the energy scattered back toward the sensor (backscattering) are received and recorded. The brightness of an object in a SAR image corresponds to its radar backscattering coefficient σ°. Because of the large dynamic range of SAR systems, the unitless σ° is normally expressed in decibels ($\sigma^\circ_{\mathrm{dB}} = 10 \log \sigma^\circ_{\mathrm{linear}}$). Backscattering signatures consisting of σ° at CHH, CHV, LHH, and LHV were extracted for rectangular polygons from Magela floodplain, using the video record as a basis for polygon location. For the April scene, the entire floodplain was inundated except for a few small islands. Floodplain polygons were initially grouped into flooded *Melaleuca* forest/woodland, aquatic macrophyte, and open water classes. For the *Melaleuca* and macrophyte classes, appearance of the polygons on CHH/LHH/CHV and CHH/LHH/LHV color composites, scatterplots of polygon medians for combinations of CHH, CHV, LHH, and LHV, and probability density functions of individual and grouped polygons were used to distinguish subclasses, which were cross-referenced with mapped plant communities and with notes on community types from the video survey. In comparing April and October signatures, a mission-

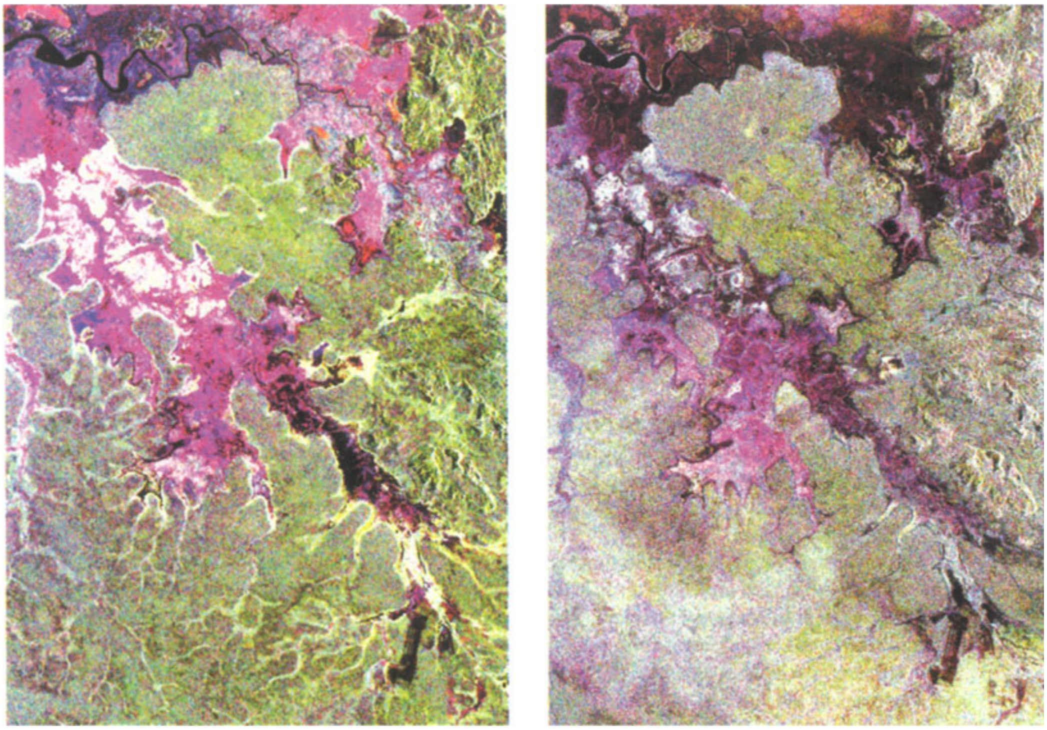

Figure 2. Color composites of Alligator River segment of SIR-C swath imaged during wet season, April 1994 (left) and dry season, October 1994 (right). CHH displayed as red, LHH as green, LHV as blue.

to-mission uncertainty of 2.2 dB for C-band and 1.3 dB for L-band was assumed, and differences lower than that were not considered significant. These values were based on estimates by Freeman et al. (1995) of pass-to-pass calibration uncertainties for the two missions, using the maximum value for both bands.

Using classes and subclasses as response variables and σ° values at the four SIR-C band and polarization combinations as predictor variables, classification rules were generated by a decision-tree model (Clark & Pregibon, 1992), and a rules-based classification was performed for the floodplain area. Because of the heterogeneity of the macrophyte and *Melaleuca* classes and the relatively large pixel size of the April SIR-C data, independent test and training polygons could not be identified for some subclasses. Each polygon was therefore divided by random sampling into separate training and test pixel sets; training pixels were used to generate decision rules, and test pixels were used in tree pruning (described below) and to evaluate classification accuracy. Training and test pixels were selected in a similar fashion for the October scene, with the addition of unflooded forest/woodland, unflooded macrophyte, and bare ground to the initial floodplain classes.

A pruning strategy was used to determine final tree size. A tree with a large number of terminal nodes N was derived from the training dataset, then nodes were successively pruned back to produce a set of trees with number of nodes n equal to 1, 2, 3, ... N. The test dataset was then classified using each of these trees, and the residual deviance (a measure of node heterogeneity) was plotted as a function of n; pure nodes, containing a single response variable, have deviance equal to zero. The deviance vs. number of nodes curve is typically reverse J-shaped, as deviance drops rapidly with the first several nodes, then more gradually, finally reaching a minimum and then increasing again as more nodes are added. The smallest n corresponding to the minimum deviance value was selected as

Table 1. SIR-C data parameters

	Space Radar Lab I Shuttle Mission STS-59	Space Radar Lab II Shuttle Mission STS-68
Swath number	117.6	117.52
Date	14 April 1994	6 October 1994
Wavelengths	5.7 cm (C-band), 24.0 cm (L-band)	5.7 cm (C-band), 24.0 cm (L-band)
Polarizations*	HH, HV	HH, HV
Center latitude, longitude	12.58° S, 132.84° W	12.56° S, 132.84° W
Incident angle range	36.7°–47.1°	35.5°–41.8°
Nominal resolution	50 m	25 m

*HH: horizontal send/receive; HV: horizontal send, vertical receive.

the optimal tree size from which to derive classification rules. Larger trees than this are overfit to the training data, as indicated by the rise in deviance at larger *n*, and result in higher misclassification rates when applied to test data.

A comprehensive characterization of backscattering response was not attempted for areas of the Magela subscene other than Magela floodplain, such as surrounding lowlands, escarpment, and portions of the East Alligator floodplain, because these areas were not surveyed at the time of the SIR-C acquisitions. For the same reason, the non-floodplain portions of the subscene were not classified. In order to estimate how well floodplain cover types could be discriminated from lowland, lowland eucalypt woodland and open areas overflown in the course of surveying the floodplain were located. Backscattering returns were evaluated as for the floodplain polygons, and the training pixels were input to the decision-tree model along with the floodplain samples. In addition, points of interest noted on color composites are discussed for the non-floodplain Magela subscene, and for the full Alligator River scene.

Results

Backscattering signatures: April

Flooded *Melaleuca* woodland and forest patches identifiable on the April video were in nearly all cases easily recognizable on the April SIR-C image. The exceptions were single trees, clumps of 2–3 trees, and larger clumps when mixed with *Nelumbo*. Because *Nelumbo* has a high L-band return, it was difficult to precisely locate tree clumps amongst *Nelumbo* on the SAR image. The flooded *Melaleuca* polygons were grouped into four types based on viewing color composites and scatterplots of polygon medians for combinations of CHH, CHV, LHH, and LHV. Variations in response between the types were most obvious on the CHV/LHH/LHV color composite. Figure 3 gives probability density functions for each *Melaleuca* type. The types are distinguishable by differences in CHV and LHV scattering, with little differentiation at LHH and none at CHH. There is a clear break between the high-LHV types (1 and 3) and low-LHV types (2 and 4) at σ°_{LHV} of about -17 dB. The differences in median σ°_{LHV} are large, with a maximum difference of 7.2 dB between types 3 and 4, and a minimum of 5.6 dB between types 2 and 3 (Table 2). The difference stated is in terms of decibels, equivalent to the ratio in linear scale. The groups split differently at CHV: CHV returns are higher than -12.5 dB for types 1 and 2, and lower for types 3 and 4.

Type 1 tended to occur in large, relatively uniform patches (Fig. 4a). Canopy cover (visually estimated from the video) ranged from about 50 to 100%, with cover greater than 70% for most stands. Defoliation was minimal in these stands. The more open type 1 stands tend to have lower LHH returns. Finlayson et al. (1989) give a canopy cover range of 10–70% for *Melaleuca* open forest and woodland on Magela floodplain. The visual estimate in this study of 100% canopy cover for the most dense stands may have been biased by the oblique viewpoint of the video.

Type 3 occurred mostly in conjunction with type 1, but in small patches. No large, uniform areas of this type were captured on the video. It differs from type 1 only in having lower CHV returns, but it could not be determined whether this was related to a species difference, to structural variation within a species

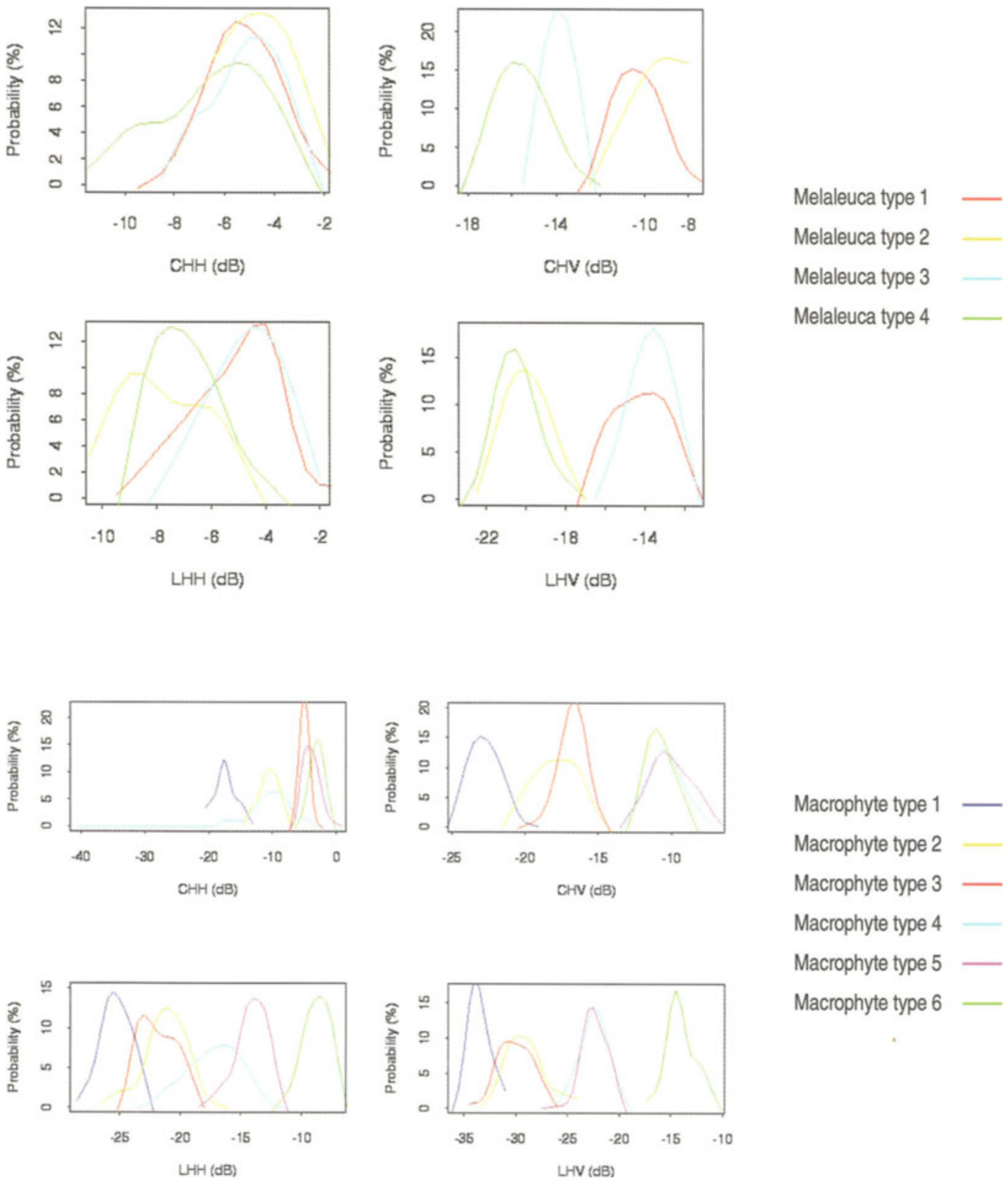

Figure 3. Probability density functions for sub-types of *Melaleuca* forest and woodland (upper), and flooded aquatic macrophyte (lower) at four SAR band-polarization combinations, April image.

Table 2. Median σ° (dB) for vegetation classes

Class	CHH	CHV	LHH	LHV	Description
April					
Water	−27.8	−23.4	−29.0	−36.2	Open water (East Alligator River)
Bare	−11.0	−17.9	−18.2	−27.7	Non-flooded lowland, bare or with sparse or senescent grass
Macro-1	−17.8	−22.7	−25.5	−34.0	Equal mix open water and flooded short/sparse macrophyte (low CHH)
Macro-2	−10.7	−17.9	−20.9	−29.5	Flooded short/sparse macrophyte with some open water (medium CHH)
Macro-3	−5.1	−16.8	−22.4	−30.3	Flooded short/sparse macrophyte (high CHH)
Macro-4	−9.8	−10.6	−16.7	−22.4	Flooded medium to high biomass macrophyte (medium CHH, high CHV) (Fig. 4d)
Macro-5	−4.2	−10.3	−14.5	−22.7	Flooded medium to high biomass macrophyte (high CHH & CHV)
Macro-6	−3.0	−11.2	−8.8	−14.5	Flooded *Nelumbo nucifera* (high CHH, CHV, LHH, & LHV) (Fig. 4c)
Woodland, non-flooded	−9.2	−14.0	−11.0	−18.1	Non-flooded lowland Eucalypt woodland (Fig. 4d, top and bottom)
Flooded *Melaleuca*-1	−5.5	−10.6	−5.5	−14.6	Flooded *Melaleuca* forest and woodland (high CHV, high LHV) (Fig. 4a)
Flooded *Melaleuca*-2	−4.7	−9.8	−8.2	−20.2	Flooded *Melaleuca* open and very open woodland (high CHV, low LHV) (Fig. 4b)
Flooded *Melaleuca*-3	−5.1	−14.2	−4.9	−13.8	Flooded *Melaleuca* forest and woodland (low CHV, hi LHV)
Flooded *Melaleuca*-4	−6.4	−15.7	−7.2	−21.0	Flooded *Melaleuca* open and very open woodland (low CHV, low LHV)
October					
Water	−28.9	−23.7	−30.5	−35.6	Open water: billabongs and East Alligator River
Bare	−13.4	−19.5	−20.3	−31.8	Non-flooded lowland & floodplain, bare or with sparse or senescent grass
Macro-4	−12.3	−9.8	−14.1	−18.0	Flooded medium to high biomass macrophyte, incl. senescent (high CHV)
Macro-5	−5.1	−12.0	−15.9	−25.0	Flooded medium to high biomass macrophyte (high CHH, medium CHV)
Macro-6	−4.4	−12.3	−10.0	−20.1	Flooded *Nelumbo nucifera* (high CHH & LHH)
Woodland, Non-flooded	−9.7	−13.4	−12.1	−18.4	Non-flooded lowland Eucalypt woodland & floodplain *Melaleuca* forest/woodland
Flooded *Melaleuca*	−5.8	−11.3	−5.4	−15.2	Flooded *Melaleuca* forest, woodland, and very open woodland

due to different environmental conditions or age, or to random canopy variability. Types 2 and 4 (the low-LHV types) also tended to occur in small patches or scattered pixels, making it difficult to relate the groups to stand characteristics seen in the video. However, in almost all cases these types corresponded to shorter, more widely spaced trees with smaller crowns (Fig. 4b) than types 1 or 3. The lower canopy cover is consistent with the low LHV returns for these two types, since volume scattering from branches is the source of LHV scattering. Stands at the low end of the 10–70% range cited by Finlayson et al. (1989) would be included in these types. Of the two types, type 4 has the lower density, often corresponding to very widely spaced trees; there is little canopy interception, and double-bounce HH returns from trunks dominate the signal. The most extensive areas of Type 4 were in back-plain swamps, where trees are defoliated and many are dead (Devonport 1993). The open stands in the deeper parts of the back-plain swamps have low CHV and LHV (type 4), while the denser stands at the border have high LHV and variable CHV (types 1 and 3). Defoliation does not appear to significantly alter the signature of these stands compared with fully foliated stands.

The correspondence between relatively homogeneous patches visible on the survey video and patterns on the SAR image was less consistent for aquatic macrophytes than for *Melaleuca* woodland. Some image patterns clearly corresponded with the video while

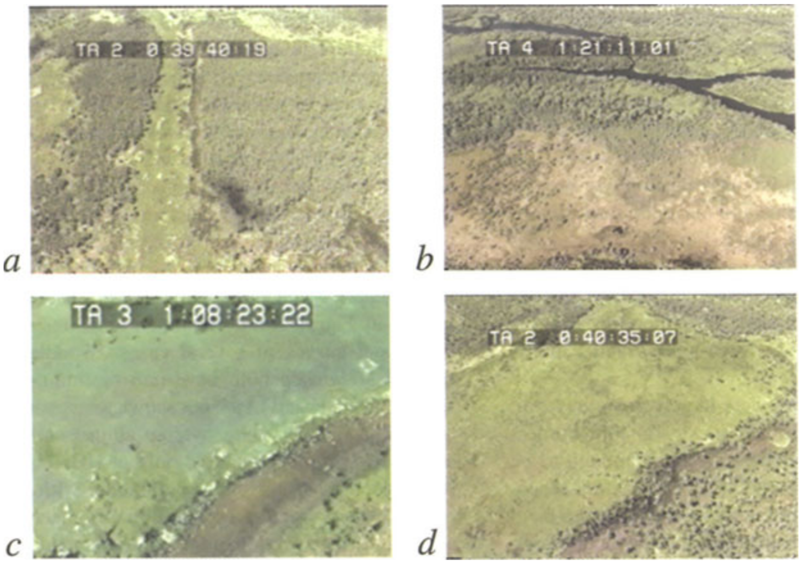

Figure 4. Video images obtained during April 1994 aerial survey of Magela floodplain: (a) high-density flooded *Melaleuca* forest and woodland, lower basin; (b) lower-density *Melaleuca* woodland, Mudginberri corridor; (c) *Nelumbo nucifera*, Magela Plain; (d) macrophyte type 4, back-plain swamp.

others did not. The spatial patterns of the numerous macrophyte communities are complex, and vary at scales on the order of a few to a few hundred meters. The 50 m resolution of the April scene is too coarse to capture all of this variation, and patterns on the SAR in some cases correspond to average returns from two or more patches that are visually distinctive on the video. In addition, some of the macrophyte communities had low biomass at the time of the April acquisition, and appeared similar to open water on the SAR image.

Probability density functions for six macrophyte types are given in Figure 3. The range in median σ° for the six types is 12 dB or more at all four combinations. The types fall into well-separated groups having low biomass (types 1, 2, and 3) and high biomass (types 4 and 5), at both CHV (low biomass returns are < -14 dB) and LHV (low biomass returns are < -26 dB). No biomass measurements were taken at the time of the flight, and the term is used here to qualitatively describe differences that were apparent on the video, related to a combination of percent canopy cover and canopy height, and that normally are correlated with biomass. The low-biomass types are three segments of a continuum of a mixture of short and/or sparse macrophyte cover with open water: type 1 is a mosaic of patches of water and sparse/short macrophyte, and appears black on the CHH/LHH/CHV color composite; type 3 is a mostly continuous cover of sparse/short macrophyte appearing bright red on the color composite, and type 2 is intermediate. The three types are most clearly separable at CHH, the band most sensitive to herbaceous stem density and height for low-biomass stands: median CHH returns for types 1, 2, and 3 were -17.8 dB, -10.5 dB, and -5.1 dB.

Type 6, consisting of homogeneous areas of *Nelumbo nucifera*, was easily recognizable on the video by its distinctive bluish-green cast (Fig. 4c). It is clearly distinguishable from the other macrophyte types by its LHV scattering (median -14.2 dB), quite high for non-woody vegetation. Median LHV returns for the other high-biomass types are -22.5 dB. LHH returns are also higher than for the other macrophyte types, with a 5.7 dB difference in median σ°_{LHH} between macrophyte types 5 and 6. The high L-band returns can be explained by *Nelumbo*'s unusual structure. The large round leaves (up to 50 cm in diameter) can be somewhat cup-shaped, and may be oriented horizontally or at an angle, with petioles up to 2 m tall; these are the likely source of the strong LHV scattering. LHH returns are probably primarily from vertically oriented pedicels, which can exceed petioles in length (Cook, 1996). The high water content of both stalks and leaves further enhances returns. The probability density functions for macrophyte type 6 are derived from *Nelumbo* sites that can be seen on the video to be free of trees, but in other parts of the floodplain *Nelumbo* occurs as understory with widely spaced *Melaleuca*.

The other two high-biomass macrophyte groups, types 4 and 5, appear blue and magenta, respectively, on the CHH/LHH/CHV image (Fig. 2). The two types are nearly identical at CHV and LHV (Fig. 3), but median σ° for type 4 is 5.6 dB lower at CHH, and 2.2 dB lower at LHH. It is not clear from the video what is causing the differences between types 4 and 5 in HH (but not HV) returns.

All macrophyte types were largely green in April, and senescence was limited mostly to thin borders at the perimeters of patches, not large enough to be treated separately in the analysis. A few larger senescent areas were noted. In Figure 4c, the brownish band between the *Nelumbo* patch and the lowland, which resembles bare ground, can be seen on the continuous video to be flooded, with apparently senescent vegetation. The backscattering signatures for these few small areas are nearly identical to that for type 3.

Areas of open water on the East Alligator River were used to represent the open water class. Backscattering response for the Alligator River differed from that for billabongs, especially at CHH and LHV. The billabongs are seen in the video to be largely clear of plant cover, and the few macrophyte patches on billabongs were avoided in polygon selection. The high returns from most pixels in the billabongs (similar to low-biomass macrophyte, but with higher σ°_{LHH} and σ°_{LHV} returns) is probably due to mixed pixel effects caused by the low resolution of the SAR relative to the width of the billabongs, most of which are oriented nearly perpendicular to the SIR-C look direction. Strong returns from double-bounce interactions between open water and vegetation at the edge of the billabongs, when averaged with low open water returns, result in edge pixels of intermediate values.

Backscattering characteristics of lowland eucalypt woodland polygons and bare ground polygons were examined to detect possible sub-classes. Variability in the eucalypt woodland, mainly related to stem density differences, was apparent on the color composites (Fig. 2). Probability density functions of potential sub-classes overlapped at all band/polarization combinations, however, so all polygons were combined into a single nonflooded woodland class. Bare ground polygons included unflooded areas with sparse or senescent grass as well as bare soil areas. No open areas off the floodplain appeared in the video to have substantial green grass cover, although some green areas could be seen in woodland gaps. All lowland eucalypt woodland areas viewed in the video had low stand densities, and none were flooded.

Probability density functions for the bare ground and lowland woodland classes are plotted with those of the floodplain classes in Figure 5. The spread in the functions shows the degree of within-class heterogeneity. The floodplain subtypes plotted separately in Figure 3 are combined here, resulting in polymodal curves in several cases. Woodland and flooded *Melaleuca* both have narrow, unimodal responses at CHH and LHH, but show greater variability at CHV and LHV; they overlap almost completely at HV, and are differentiable only at HH. The curve for flooded macrophytes is polymodal with a large range at every band. The low-biomass macrophytes overlap the bare ground class, while the macrophytes with higher biomass overlap the woodland and flooded *Melaleuca* classes. LHH is the only band at which macrophytes and flooded *Melaleuca* can be separated (except for *Nelumbo*, which has a similar distribution to *Melaleuca*). At CHH, all classes other than water overlap to a significant extent. Median σ° is higher for flooded than nonflooded woodland for all four types of flooded woodland at CHH and LHH, but flooded woodland types 2, 3, and 4 have lower $\sigma^\circ_{\mathrm{CHV}}$ or $\sigma^\circ_{\mathrm{LHV}}$ than nonflooded woodland. The differences in median σ° between flooded and nonflooded woodland for types 1–4 range from 2.8 to 4.5 dB at CHH, and 2.8 to 6.1 dB at LHH.

Backscattering signatures: October

Nonflooded areas of the floodplain were difficult to distinguish from flooded areas on the October video except for sites where water was obvious in openings in the macrophyte or tree cover. Sun glint was much less apparent than on the April video, owing to hazy conditions or unfavorable sun angle. Some of the smaller open water areas had a high sediment content, closely resembling bare soil or senescent macrophyte in appearance on the video. Since few *Melaleuca* stands other than those at the floodplain/lowland interface could definitely be identified as nonflooded, training data for nonflooded forest and woodland were taken both from *Melaleuca* stands at floodplain borders, where the area to the floodplain side of the stand could be seen to be dry, and from adjacent lowland eu-

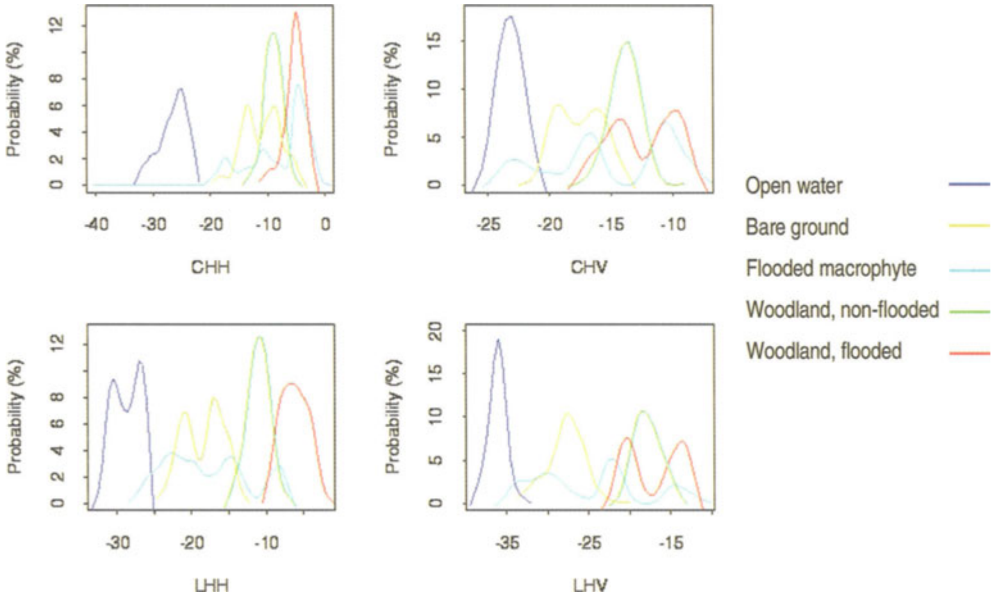

Figure 5. Probability density functions for categories used in cover-state classification, April image.

calypt stands. The nonflooded woodland class is thus a mixture of lowland and floodplain woodlands.

The maximum decrease from April to October in median $\sigma^\circ_{\mathrm{HH}}$ for an individual stand was 5.8 dB at CHH and 6.4 dB at LHH; cross-polarized differences were smaller (2.3 and 2.8 dB decreases at CHV and LHV). For other stands, October median $\sigma^\circ_{\mathrm{CHH}}$ was 2.3 dB lower than in April, and differences at the other band and polarization combinations were within the mission-to-mission calibration uncertainties. Some *Melaleuca* stands that remained flooded in October had higher returns in October than April. For stands near the centerline of back-plain swamps, $\sigma^\circ_{\mathrm{CHH}}$ was unchanged, but σ° increased by 2.5, 1.6, and 3.7 dB at CHV, LHH, and LHV, respectively. This change is consistent with a decrease in water level, exposing more of the tree trunk and/or lower crown. Increased returns from flooded *Melaleuca* could be seen in other parts of the floodplain as well, mainly in backswamps.

Green macrophyte areas were largely limited in October to creek channels, billabong fringes, and backplain swamps. Some senescent macrophyte areas remained flooded; these were included in the flooded macrophyte class. Non-flooded senescent macrophytes were included in the bare class, along with lowland open ground. The April flooded macrophyte types with the lowest biomass (types 1 and 2) were senescent in October and no longer flooded. Besides these areas, which correspond mostly to *Pseudoraphis* grassland, other floodplain areas included in the bare class included burn scars. These were often at floodplain margins, extending from lowland woodland or open areas to the edge of areas that remained flooded.

Macrophyte areas with signatures similar to the April types 4, 5, and 6 remained in October, as did small groups of pixels similar to type 3. These small groups were combined into the October type 5 class, resulting in three flooded macrophyte types for October. All three macrophyte types occurred in smaller-sized patches than in April. Median σ° values for type 5 in October are very close to the average for type 3 and 5 in April (Table 2). There are significant differences between April and October for types 4 and 6, however. For type 6 (*Nelumbo nucifera*), median $\sigma^\circ_{\mathrm{LHV}}$ dropped by 5.6 dB in October, while differences in returns at the other bands/polarizations are within the calibration uncertainty. The decrease in $\sigma^\circ_{\mathrm{LHV}}$, but not in $\sigma^\circ_{\mathrm{LHH}}$, suggests that vertical elements of the *N. nucifera* canopy, such as pedicels, were present in both April and October, whereas elements oriented at an angle (leaf blades and petioles) were present in April but not October.

The trend in $\sigma°_{LHV}$ for type 4 was opposite to that of type 6: median LHV returns increased by 4.4 dB between April and October (Table 2), and individual stands had much larger increases. A patch near the center of the same type 4 area shown in Figure 4c had an increase in median $\sigma°_{LHV}$ of 8.2 dB, to -16.4 dB. This high $\sigma°_{LHV}$ is remarkable since the macrophyte cover appears on the video to be nearly uniformly senescent. One explanation for the high LHV returns would be that senescent leaves (seen on the video) formed a mat over green grass or sedge culms. *Hymenachne acutigluma* and *Eleocharis dulcis*, components of the *Hymenachne-Eleocharis* community associated with type 4, both have culms 1 m or more in length; *H. acutigluma* culms are described by Cook (1996) as floating, creeping, or ascending, and often softly spongy (aerenchyma-filled) rather than hollow, which would increase SAR reflectivity.

For the woodland class, differences between April and October were well within the calibration uncertainty at both bands and polarizations. The same is true for water, except that $\sigma°_{LHH}$ decreased in October by 1.5 dB, slightly more than the L-band calibration uncertainty. For the bare class, however, $\sigma°$ decreased at all four combinations; the largest difference was -4.1 dB at LHV.

Image classification

The overall misclassification rates for test pixels were 9.4% in April and 6.2% in October; Table 3 shows errors by class. The principal sources of error for the wet-season classification were (1) the similarity in backscattering between bare ground and low-biomass macrophyte, causing 9.5% of bare ground pixels to be labeled as macrophyte; (2) misclassification of 7.3% of bare ground as non-flooded woodland, resulting from several pixels with anomalously high LHV returns in the test pixel set; (3) misclassification of 13.9% of nonflooded woodland as macrophyte – these appeared to correspond to more open areas within the woodland, with lower woody biomass; and (4) misclassification of flooded woodland as macrophyte (7.7%), owing to similarity of backscattering response between *Nelumbo* and flooded *Melaleuca*.

Misclassification of bare pixels as macrophyte or woodland was lower in October. The absence of short or sparse macrophyte stands improved the separability of the bare and macrophyte classes; lower soil moisture and increased grass senescence improved the separability of the bare and woodland classes. The number of macrophyte pixels misclassified as non-flooded woodland increased by about 7% in October. This error can be attributed mostly to increased $\sigma°_{LHH}$ and $\sigma°_{LHV}$ for macrophyte type 4, which decreased its separability from woodland; also, type 4 accounted for a larger percentage of the overall macrophyte class since the low-biomass macrophyte types did not occur in October. The number of flooded *Melaleuca* pixels misclassified as macrophyte decreased from 7.7% in April to 0% in October. This improvement occurred because $\sigma°_{LHH}$ and $\sigma°_{LHV}$ decreased in October for *Nelumbo*, increasing its separability from *Melaleuca*.

The classified, masked floodplain images for April and October are shown in Figure 6 with the vegetation map for comparison. On the April image, flooded Melaleuca areas (white on the classified scene) correspond well to the mapped areas of *Melaleuca* open forest and woodland. Most areas mapped as *Melaleuca* open woodland, including cross-hatched areas on the map, appear on the classified image as a speckled mixture of *Melaleuca* and macrophyte, as opposed to the more solid patches of pixels corresponding to the *Melaleuca* open forest and woodland areas. The only mapped *Melaleuca* polygons not well matched in the classification are an open forest and woodland area opposite Leichhardt Billabong and an open woodland area at the mouth of a back-plain swamp north of Nankeen Billabong; both of these were classified as macrophyte, with few or no *Melaleuca* pixels interspersed. These discrepancies may result from the SIR-C resolution being too coarse for very widely spaced trees to dominate backscattering from the pixels in which they are situated. The other discrepancy between classified and mapped *Melaleuca* is that many pixels classified as macrophyte type 6 (*Nelumbo)* occur in mapped *Melaleuca* locations. These pixels are not apparent in Figure 6 since they occur in small patches. The error is to be expected, based on the similarity in backscattering between the two classes.

In the case of *Melaleuca*, vegetation community (the mapped feature) translates directly to vegetation structure (the feature that was sensed by the SAR and then classified), because *Melaleuca* is the only major woody community present on the floodplain. Furthermore, barring unusual events the location of the *Melaleuca* stands is stable over a time scale of decades. For macrophytes, however, community differences may or may not translate to structural differences: the relationship between structure and community changes, depending on phenologic stage, as does the spatial extent of some communities. Never-

Table 3. Contingency tables for classified images (percent)

	Water	Bare	Macrophyte	Woodland, non-flooded	Woodland flooded
April					
Water	98.0	0	2.0	0	0
Bare	0	83.2	9.5	7.3	0
Macrophyte	0.4	0	96.7	0	2.9
Woodland, non-fl.	0	0.1	13.9	83.3	2.7
Woodland, fl.	0	0	7.7	4.8	87.5
October					
Water	95.4	4.6	0	0	0
Bare	2.0	93.7	4.3	0	0
Macrophyte	0	1.3	87.7	7.3	3.6
Woodland, non-fl.	0	0	14.7	83.0	2.3
Woodland, fl.	0	0	0	2.0	98.0

theless, macrophyte types classified in the April scene do appear in many cases to be coincident with mapped communities.

In general, the low-biomass types (1, 2, and 3) corresponded to areas mapped as *Pseudoraphis* grassland, and the non- *Nelumbo* high-biomass types (4 and 5) corresponded to the other grassland and sedgeland types. Macrophyte type 1 occurred almost exclusively in *Pseudoraphis* map units on the western side of the upper basin. Macrophyte type 2 occurred throughout the *Pseudoraphis* areas of the upper basin, and also in back-plain swamp areas mapped as *Hymenachne* grassland and *Nelumbo* swamp. There are no distinct patches of macrophyte type 3, only small groups of pixels scattered throughout macrophyte areas. Although macrophyte type 3 does not seem to be a distinct class on the Magela floodplain, it occurs in large homogeneous patches on the East Alligator floodplain. Uniform patches of macrophyte type 4 pixels on the classified image correspond closely to areas mapped as *Hymenachne-Eleocharis* swamp, and macrophyte type 6 patches occurred in areas mapped as *Nelumbo*, mostly on the Magela Plain. However, a few areas mapped as *Hymenachne-Eleocharis* swamp, and many of the areas mapped as *Nelumbo*, were classified as a mixture of macrophyte types 4 and 5. The other grassland and sedgeland communities mapped (*Oryza* grassland, *Hymenachne* grassland, mixed grassland/sedgeland, and *Eleocharis* sedgeland) also were classified mainly as macrophyte types 4 or 5.

Thirty-seven percent of masked floodplain was classified in April as flooded *Melaleuca*; this compares with the figure of 40% given by Finlayson et al. (1989). Only 0.02% of the floodplain was classified as water. This is partly because of the problem of mixed pixels along the borders of billabongs, but even with these pixels included, the open water area would be less than 1%. All mapped macrophyte communities were represented in more than one macrophyte type in the classification, so areas covered by individual macrophyte communities could not be compared with classified macrophyte types.

In October, flooded *Melaleuca* is restricted mainly to backswamps, the lower basin, and the Mudginberri Corridor (Fig. 6). *Melaleuca* stands along the floodplain border are mostly dry. Most of the flooded macrophyte areas are in backswamps, particularly the Magela Plain and the backswamps to the north of it. Most of these areas are mapped as *Nelumbo* swamp, *Hymenachne-Eleocharis* swamp, or *Hymenachne* grassland on the vegetation map. Areas mapped as *Pseudoraphis* are largely dry, and *Oryza* and mixed grassland/sedgeland areas are a mixture of flooded and dry. The area of open water on the floodplain increased, as the emergent macrophytes that covered some of the more permanently flooded channels and backswamps in April had senesced and were no longer emergent in October. The proportion of *Melaleuca* woodland that was flooded decreased from 100% in April to 20% in October.

The low misclassification rates for test data and the correspondence of vegetation patterns on the classi-

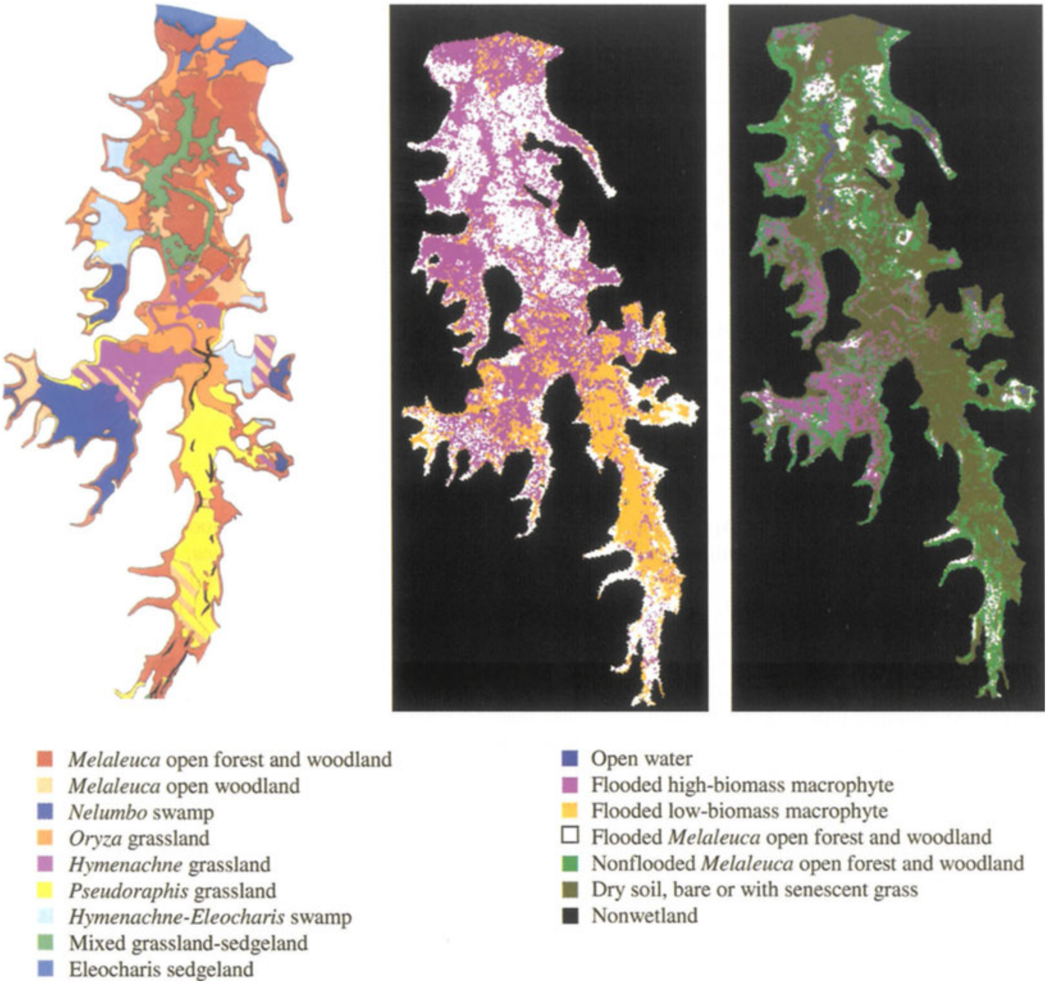

Figure 6. Left to right: vegetation map of Magela floodplain (Finlayson et al., 1989); classified SIR-C image, wet season, April 1994; classified SIR-C image, dry season, October 1994.

fied images and vegetation map indicate the suitability of multi-frequency SAR data for detecting flooding, phenologic state, and in some cases plant community for Magela floodplain. The similarity of Magela floodplain to the other imaged portions of the East and South Alligator systems suggests that these results can be extended to other floodplain sites in the Kakadu region as well.

Multitemporal color composite

An alternative way of extracting information on the dynamics of vegetation and flooding is to treat the two dates as a combined dataset rather than classifying them separately. This approach was not taken because of uncertainty introduced by the difference in resolution between the two dates. However, the striking patterns evident on a two-date color composite of the Magela scene (Fig. 7) indicate that multitemporal SAR data provides a wealth of information for both floodplain and lowlands. In this color composite, $\sigma^\circ_{\mathrm{CHV,Oct}} - \sigma^\circ_{\mathrm{CHV,Apr}}$ is displayed as red, $\sigma^\circ_{\mathrm{LHH,Oct}} - \sigma^\circ_{\mathrm{LHH,Apr}}$ as green, and $\sigma^\circ_{\mathrm{LHV,Oct}} - \sigma^\circ_{\mathrm{LHV,Apr}}$ as blue. *Pseudoraphis* grassland, which was in an early emergent state in April, is conspicuous as bright white areas in the Magela upper basin. Blue and bluish-white areas, which had higher returns in October, particularly at LHV, correspond very well to *Hymenachne-Eleocharis* swamp, *Hymenachne* grassland, and *Eleocharis* swamp, and to some areas of mixed grassland/sedgeland. Areas similar to this can be seen in backswamps of the East Alligator River at the upper right. *Oryza* areas in the lower basin and backswamps appear dark green, indicating higher $\sigma^\circ_{\mathrm{LHH}}$ in October. *Nelumbo*-dominated areas on the Magela Plain appear reddish green. The channel of Magela Creek is more conspicuous on this image than at either of the single dates: the wider parts of the channel in the lower basin are dark (open water in October), and the channel banks are bordered with grassland/sedgeland. A band of dark red traces the floodplain borders, corresponding to woodland areas that changed from flooded to nonflooded status. The same signature extends into the lowlands, clearly delineating lowland drainageways that feed onto the floodplain. For some backswamps, inundated woodland extended well beyond the floodplain boundary in April. The signature of the extensive dark regions on the East Alligator floodplain is not found on Magela floodplain. Some of these areas are bright on the April scene, with a signature resembling non-flooded woodland, but the dark appearance in October makes it likely that this vegetation is herbaceous rather than woody.

Conclusions

This study assessed the usefulness of multifrequency SAR for monitoring flooding and vegetation on seasonally inundated floodplains in Australia. Because oblique aerial surveys rather than ground sampling or vertical aerial photography were used for field verification, quantitative relationships between stand structural parameters and radar backscattering could not be evaluated. However, general vegetation structure and flooding state were documented for a large area with a wide variety of vegetation types, making it possible to describe the range in σ° to be expected for both woody and herbaceous vegetation, in dense or open stands, and in flooded or nonflooded states. Although many questions remain, these results strongly indicate the usefulness of multifrequency SAR in the following areas: (1) Mapping of woody and herbaceous vegetation in flooded and nonflooded states. For the October scene, where all five vegetative/hydrologic classes were present on the floodplain, classification accuracies were greater than 83% for all classes. Correspondence between areas classified as *Melaleuca* with mapped areas of *Melaleuca* on the vegetation map support the accuracy of the woody/herbaceous classification. Flooded herbaceous and woody vegetation were well separated at LHH: with the exception of *Nelumbo nucifera*, median $\sigma^\circ_{\mathrm{LHH}}$ for macrophytes was at least 6 dB lower than for *Melaleuca* on both dates. The difference in median $\sigma^\circ_{\mathrm{LHH}}$ between nonflooded woodland and the various flooded *Melaleuca* classes ranged from 2.8 to 6.7 dB, and the difference for all *Melaleuca* classes considered together was 4.6 dB in April and 6.7 dB in October. These large differences, consistent under both late wet and late dry season conditions, indicate that mapping of vegetative/hydrologic classes can be accomplished with high accuracy using multifrequency SAR. (2) Discriminating among aquatic macrophyte communities. While further study is required to quantify the relationship between macrophytes subtypes and structural or floristic measures of interest, in several cases there is a strong correspondence of macrophyte types with mapped communities: type 6 with *Nelumbo nucifera*, types 1–3 with *Pseudoraphis* grassland, and type 4 with *Hymenachne-Eleocharis* swamp. The correspondence between classified and mapped communities results from differences in canopy structure related both to phenologic state and to species morphology. *Pseudoraphis* grasslands are distinctive because they are in an early emergent state in April, with a shorter and sparser canopy relative to the other communities. *Nelumbo* is distinctive because of its large stalks and leaves (lower accuracy rates for *Nelumbo* result from confusion with *Melaleuca*, not with other macrophyte types). For *Hymenachne-Eleocharis* swamp, $\sigma^\circ_{\mathrm{CHV}}$ is higher relative to $\sigma^\circ_{\mathrm{CHH}}$ compared with other macrophyte types, in both April and October; this difference is probably related to stem orientation. (3) Monitoring phenologic change in macrophyte communities. Several communities exhibited marked differences in SAR response between April and October. Median backscattering from *Pseudoraphis* grasslands south of Leichhardt Billabong increased by 7.4, 9.2, 11.1, and 7.3 dB at CHH, CHV, LHH and LHV; for a typical *Hymenachne-Eleocharis* swamp, CHH returns were unchanged between April and October, while CHV returns decreased by 2.6 dB, and LHH and LHV returns increased by 8.1 and 7.7 dB; and for an area of *Oryza* grassland near the East Alligator floodplain, σ° decreased by 5.7, 7.2, and 3.7 dB at CHH, CHV,

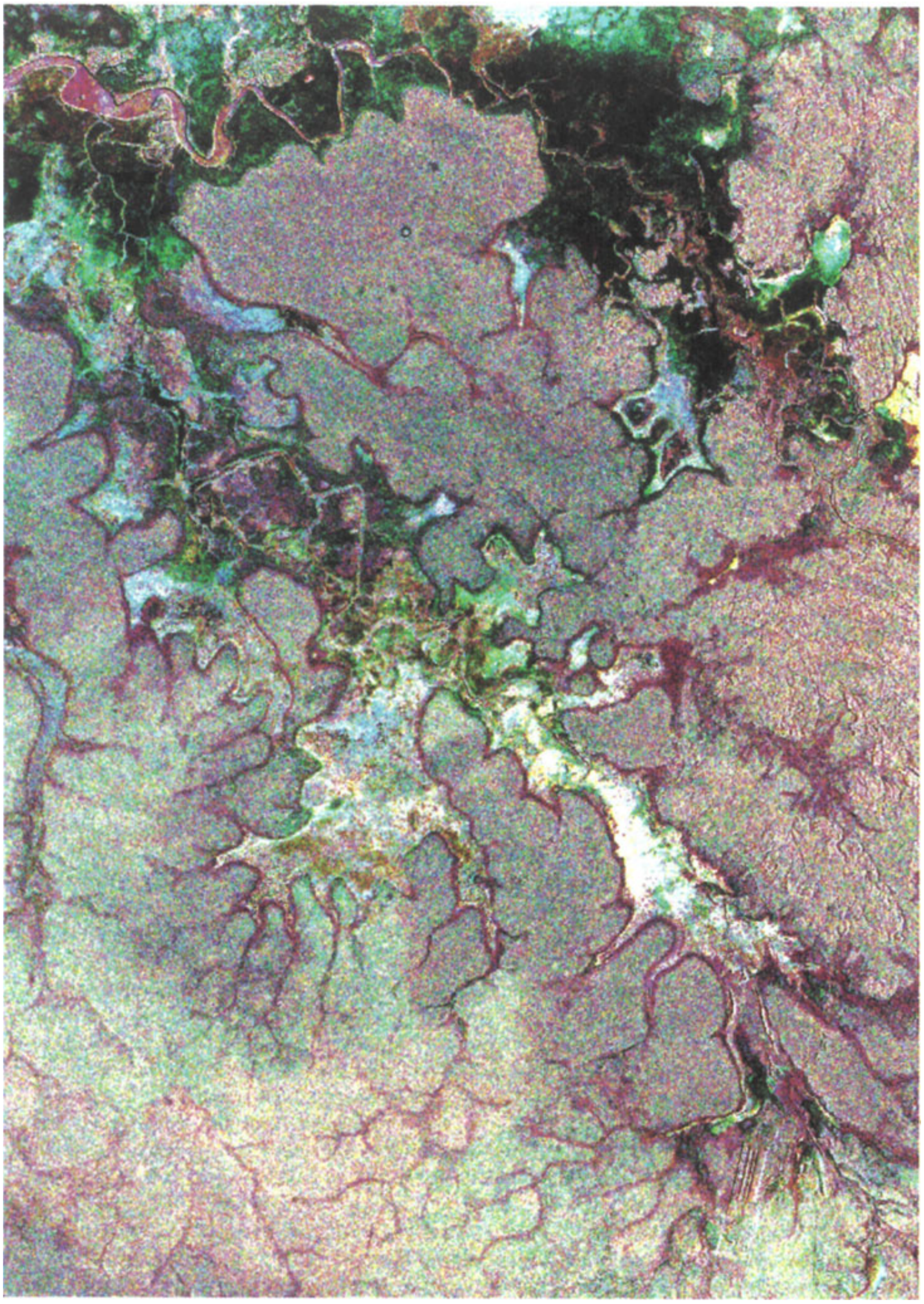

Figure 7. Dual-season color composite, Alligator River SIR-C scene. $CHV_{Oct} - CHV_{Apr}$ displayed as red, $LHH_{Oct} - LHH_{Apr}$ displayed as green, $LHV_{Oct} - LHV_{Apr}$ displayed as blue.

and LHV but increased by 2.1 dB at LHH. In general, multi-temporal variability between macrophyte communities was higher at C-band than at L-band. The exception was *Nelumbo*, for which median $\sigma^{\circ}_{\mathrm{LHV}}$ was 5.6 dB lower in October. The change for *Pseudoraphis* results from increased height and canopy cover. Based on the appearance of the other communities on the video, the changes seem to be related mainly to senescence, which causes differences in stem and stalk angles, and affects the balance of canopy attenuation by leaves and reflections from stems and stalks.

From the above results for mapping wetland communities, inundation state, and phenology at Magela Creek, we conclude that SAR remote sensing may be used for a variety of ecological and hydrologic applications in subcoastal floodplain environments of northern Australia. Because of the variability in stand structure, phenology, and inundation state in these wetland plant communities, best results would be obtained by using a configuration similar to SIR-C mode 11, i.e. C- and L-band, dual-polarization. Currently, dual-polarized C-band data can be obtained with the Envisat ASAR, but there are no L-band SAR satellites. After the planned launch of NASDA's ALOS satellite in 2004, dual-frequency, dual-polarization data sets should be obtainable by combining ASAR with dual-polarization data from the ALOS PALSAR. The seasonal variation in backscattering for the Magela floodplain communities suggests that using multitemporal data, it will be possible to discriminate among communities that cannot reliably be distinguished using a single date.

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Impact of fish predation on coexisting *Daphnia* taxa: a partial test of the temporal hybrid superiority hypothesis

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Key words: Daphnia, temporal hybrid superiority hypothesis, fish predation, co-existence

Abstract

Fish predation was tested as a factor mediating the coexistence of *Daphnia* taxa in the shallow, hypertrophic Lake Blankaart. Naturally co-occurring populations of *D. galeata* and the hybrid *D. galeata x cucullata* were subjected to different levels of fish predation in in situ enclosures. In control enclosures without fish, the largest taxon *D. galeata* rapidly became dominant over the intermediate sized *D. galeata x cucullata*, mainly as a result of higher birth rates. In enclosures with fish, population densities of *D. galeata* dropped relative to *D. galeata x cucullata*, due to higher mortality rates. These results are in concordance with the 'temporal hybrid superiority hypothesis', and can be explained by a higher vulnerability of the large and more conspicuous *D. galeata* to the size selective predation exerted by visually hunting planktivorous fishes. After approximately one month, however, population growth rates of *D. galeata* and *D. galeata x cucullata* in the enclosures with fish converged, due to a relative reduction in the mortality rate of *D. galeata*. This suggests that, in the presence of fish, *D. galeata* may co-exist with hybrids due to a decrease in its relative vulnerability to visual predation with time. Indeed, both *D. galeata* and the hybrid showed strong reductions in adult body size in the enclosures with fish, but this size reduction tended to be stronger in *D. galeata* than in *D. galeata x cucullata*. In addition, turbidity increased in the enclosures with fish and may additionally have reduced the relative advantage of *D. galeata x cucullata* with regard to mortality caused by visual predation.

Introduction

Hybrid zones can be defined as regions in which genetically distinct populations meet, mate and produce hybrids (Barton & Hewitt, 1985). Hybrid zones are most often envisaged as 'tension zones', where hybrid zones are maintained by a balance between dispersion and recurrent hybridisation on one hand and endogenous selection against the less fit hybrids on the other hand (Barton & Hewitt, 1985, 1989; Bierne et al., 2002). In some species complexes and under certain conditions, however, fitness of hybrids is believed to be equal to or higher than in the parental species. In such cases, the occurrence of hybrid zones is best explained by an interaction between selection conditions and taxon specific characteristics ('bounded hybrid superiority'; Moore & Koenig, 1986; Good et al., 2000). It may be expected that hybrid superiority is more easily accomplished in cyclic parthenogenetically reproducing organisms, because they can establish clonal lineages which enable them to circumvent problems associated with sexual reproduction (hybrid breakdown).

In the cyclic parthenogenetically reproducing genus *Daphnia*, hybrids are known to coexist with parental species in many populations (Taylor & Hebert, 1992, 1993; Schwenk & Spaak, 1995). A survey by Schwenk & Spaak (1995) has indicated that in the *D.*

galeata x cucullata species complex, at least 40% of the populations contain hybrids, while this proportion amounted to almost 80% in the *D. galeata mendotae x rosea* complex (Schwenk, 1997). There are field data as well as laboratory experimental evidence supporting the idea of hybrid superiority as an explanation for hybrid maintenance in *Daphnia* populations. Several *Daphnia* populations are temporally dominated by hybrid taxa (Spaak & Hoekstra, 1995, 1997), and some populations are even known to be almost exclusively composed of hybrids (Taylor & Hebert, 1992, Spaak & Hoekstra, 1995). The temporal superiority of hybrid clones suggests niche differentiation between hybrids and parental species. Indeed, several studies indicate ecological differences between hybrid and parental taxa. Spatial segregation of hybrids and parental taxa has been reported for several natural populations (Müller & Seitz, 1993; Taylor & Hebert, 1993). Hybrid taxa have also been shown to differ in life history strategy from their parental species, with most but not all life history traits being intermediate to those of the parental species (Weider, 1993; Boersma & Vijverberg, 1994a, Spaak & Hoekstra, 1995). Boersma & Vijverberg (1994a) compared life histories of the taxa *D. galeata*, *D. galeata x cucullata* and *D. cucullata* raised at different food levels. Their results suggested a competitive inferiority of hybrids to *D. galeata* under food limiting conditions, while the relative performance of hybrids was highest at high food levels.

Despite clear-cut differences for ecologically relevant traits between parental species and hybrids of *Daphnia* species complexes, it has proven difficult to relate the relative success of hybrids in field populations to environmental factors. In a field study (Boersma & Vijverberg, 1994b), values of Standard Carbon Content (SCC), a measure of animal condition, were highly correlated between hybrid and parental taxa. Boersma (1995) found negative correlations between the fecundity of parental and hybrid taxa, suggesting the existence of 'hemispecific competition' (i.e. competition of hybrids with their parental species), but such competition seemed to have only a limited effect on population dynamics. A multivariate analysis of an extensive dataset on 31 lakes sampled during two different seasons yielded associations between environmental variables and the relative abundance of parental and hybrid taxa in the *D. galeata x cucullata* complex (Schwenk, 1997), but no causal relationships were apparent. The most convincing mechanistic explanation for the superiority of hybrids in natural populations was proposed by Spaak & Hoekstra (1995, 1997). In a laboratory life table experiment, Spaak & Hoekstra (1995) showed that the hybrid *D. galeata x cucullata* may combine the high reproductive output of the largest parental species, *D. galeata*, with the small body size of *D. cucullata*. They argued that such a life history strategy might be adaptive under strong size selective fish predation. Fish predation is positively size selective and shows large seasonal fluctuations, and as such may explain the temporal superiority of hybrids in a lake.

Spaak & Hoekstra (1995) obtained their results under standardised laboratory conditions. Under natural field conditions, however, several factors such as the presence of infochemicals, low food quality, toxic algae or pesticides may have a differential impact on the success of *Daphnia* taxa. Moreover, in the life table experiments of Spaak & Hoekstra (1995), *Daphnia* populations were not directly exposed to fish predation, but their relative ability to cope with fish predation was inferred from data on body size and reproduction. Yet, the hypothesis that fish predation may determine the relative success of taxa within a *Daphnia* species complex was corroborated by the results of a descriptive field study (Spaak & Hoekstra, 1997), in which year-to-year variation in fish predation was associated with changes in the relative abundance of hybrids and their parental species.

In Lake Blankaart, the hybrid *D. galeata x cucullata* coexists with its larger parental taxon *D. galeata*. The small *D. cucullata* is rarely observed in the lake. In this study, we set out to investigate the impact of fish predation on the coexistence of *Daphnia* taxa in Lake Blankaart. More specifically, we wanted to test the hypothesis that size selective fish predation is a major factor explaining temporal superiority of *Daphnia* hybrids under natural conditions. In addition, we aimed to investigate to what extent, and by which mechanisms, the parental taxon *D. galeata* may coexist with hybrids under conditions that favour hybrids. With this in mind, we carried out an enclosure experiment in which a natural zooplankton community, containing *D. galeata* and the hybrid *D. galeata x cucullata*, was exposed to three levels of fish predation.

Materials and methods

Enclosure experiment

In the shallow, hypertrophic Lake Blankaart (Woumen, Belgium), six cylindrical enclosures, as described by Van der Werf et al. (1987), were filled with approximately 3 m^3 of unfiltered lake water during early spring (28th of April, 1996, Day 0). On Day 9, *Leuciscus idus* with standard body length ranging between 5.3 and 7.5 cm were randomly assigned to four of the six enclosures. Two enclosures ('MF1' and 'MF2') were inoculated with two fishes each, while two other enclosures ('HF1' and 'HF2') were inoculated with six fishes each. The remaining enclosures were kept fishless ('NF1' and 'NF2'). *Leuciscus idus* is a zooplanktivore that is not native to Lake Blankaart. We chose to use this species because a previous attempt to stock enclosures with fish from the lake had failed due to high mortality. Rather than to test for predation by a specific fish species, we assumed that planktivory exerted by *L. idus* may serve as a general model for size selective fish predation.

Starting on the second day, the zooplankton of each enclosure was sampled using a Schindler-Patalas plankton trap (volume: 12 l; mesh size: 65 μm; samples were taken in the central zone of each enclosure). Two samples were taken at each of two depths (0.4 m and 1 m), pooled and fixed with a 5% sucrose formalin solution. Total chlorophyll-*a* was spectrophotometrically determined. Water transparency was measured by a Secchi-disk. During the first month, the enclosures were sampled every four days. Sampling was then reduced to once a week for one more month.

Dominant zooplankton groups (*Daphnia*, *Bosmina*, *Chydorus* and cyclopoid copepods) were counted in all samples. The size distribution of daphnids was determined by measuring at least 30 individuals from each sample. The calculation of zooplankton biomass was based on individual body weights that were estimated using published body length-dry weight relationships (Botrell et al., 1976). In addition, samples were screened for egg-bearing *Daphnia*. Egg-bearing *Daphnia* individuals were identified to species, with the taxa of the *D. galeata x cucullata* species complex separated by the morphological criteria of Flössner & Kraus (1986). As taxon discrimination of hybridising taxa may be problematic, we first evaluated whether the morphological criteria given by Flössner & Kraus (1986) were applicable to the *D. galeata x cucullata* population of Lake Blankaart. Sixteen clones from Lake Blankaart were determined as *D. galeata*, hybrids and *D. cucullata*, using morphological criteria. The clones were subsequently analysed by allozyme electrophoresis, using sAAT as a diagnostic locus (Wolf & Mort, 1986). All of the individuals had been allocated to the right taxon, showing that morphological assessment is reliable for taxon discrimination in this species complex. The body length of the egg-bearing females was measured and the number of eggs per individual was counted. Brood pouches were checked on the occurrence of dead eggs following Boersma & Vijverberg (1995). When possible, a minimum of at least 30 individuals per species was thus analysed in each enclosure and sampling day.

For each *Daphnia* taxon in each enclosure at each sampling day, the exponential population growth g since the introduction of fish was calculated as:

$$g = \ln(N_t) - \ln(N_0),$$

with N_t and N_0 the total population size at sampling time t and at the day before the introduction of fish (Day 8), respectively. As juveniles of hybrids could not be discerned from juveniles of parental species on a routine basis, estimates of total population density of each *Daphnia* taxon were obtained by extrapolating the relative abundance of the *Daphnia* taxa in the egg-bearing adult subpopulation to the whole population.

For each time interval between sampling dates, the instantaneous rate of population increase (r) was calculated for each *Daphnia* taxon in each enclosure:

$$r = \frac{\ln N_2 - \ln N_1}{t_2 - t_1},$$

with N_1 and N_2 the total population size at sampling times $t1$ and $t2$.

The birth rate b during each time interval was estimated by the Edmondson-Paloheimo equation (Paloheimo, 1974):

$$b = \frac{\ln(E+1)}{D},$$

with D the egg development time in days and E the average number of eggs per animal in the population, corrected for degenerated eggs. The egg development time (D) was determined at 15 °C for each *Daphnia* taxon in life table experiments using water of Lake Blankaart (S. Declerck, *unpublished*). D-values obtained were 3.74, 3.70 and 2.33 days for *D. galeata*, *D. galeata x cucullata* and *D. parvula*, respectively (no *D. cucullata* were observed). As the enclosure bags were solid, there was no contact between lake water and water from the enclosures. In the absence of migration, r

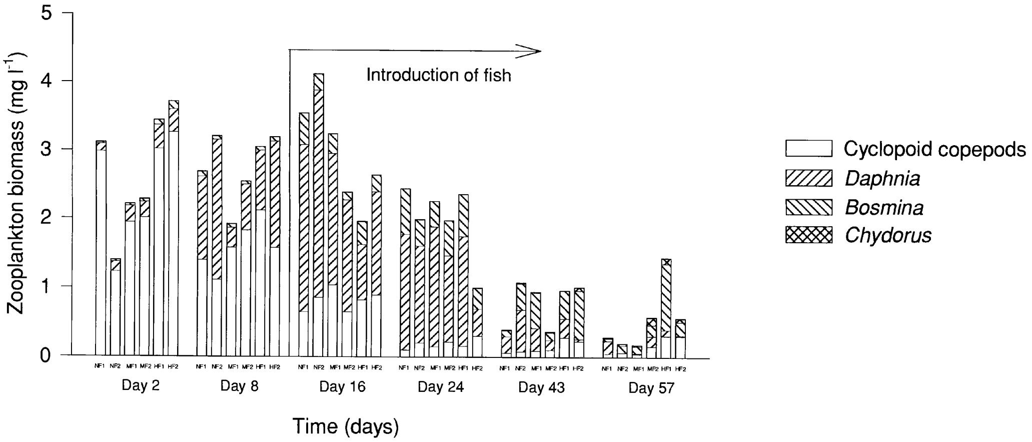

Figure 1. Changes in zooplankton biomass (mg l^{-1}) and relative contribution of main zooplankton taxa in the enclosures with time. NF1 and NF2: enclosures without fishes (No Fish); MF1 and MF2: enclosures with two fishes (Medium Fish); HF1 and HF2: enclosures with six fishes (High Fish). Fishes were introduced in MF- and HF-enclosures at Day 9. The vertical line indicates the moment of the fish introduction in these enclosures.

$= b - d$, with d the death rate in the population. Death rate (d) during a time interval was thus estimated as the difference between b and r.

Statistical analysis

For the time interval before the introduction of fish, six replicate values for r, b and d were obtained for each taxon, and comparisons between each pair of taxa were made with dependent T-tests. For the period following the introduction of fish, two-way Repeated Measures ANOVA's were carried out on population growth g, mean adult body length and mean fecundity. In these analyses, we tested for the effect of the factors 'Fish', 'Taxon' (*D. galeata* and *D. galeata x cucullata*) and 'Time' (Day 8 to Day 52). Days 57 and 65 were not included in the analyses because of the disappearance of *D. galeata* and hybrids in enclosure NF2 at the end of the experiment. Chlorophyll-*a* was analysed with a one-way Repeated Measures ANOVA, testing for the effect of 'Fish' and 'Time'. ANOVA-results were further explored by *post hoc* Tukey HSD-tests.

The patterns behind the population dynamic parameters of *D. galeata* and the hybrids showed large differences between the first period after the introduction of the fish (until Day 24) and the period following Day 24. Two separate analyses for each of both periods were therefore performed for the birth rate b and death rate d of *D. galeata* and the hybrids. For the sake of simplicity in the presentation of the results, the effect of the factor 'Time' on b and d in each of the periods will not be considered and the results of two-way MANOVA's (Multivariate Analysis of Variance), testing for the effect of 'Taxon' and 'Fish' on b and d, will be presented here as an alternative to Repeated Measures ANOVA.

Results

Zooplankton composition and biomass

At the start of the experiment (Day 2), the zooplankton community biomass was dominated by cyclopoid copepods (*Acanthocyclops robustus*) (Fig. 1). Between Day 2 and Day 8, the time interval preceding the introduction of fish, a strong increase in the biomass of Daphnids was observed. From Day 16 onwards, a steady decrease in total zooplankton biomass was observed in all enclosures. The share of cyclopoid copepods decreased, while *Bosmina* became more important. A reduction in biomass was also observed for *Daphnia*. The taxon composition of the *Daphnia* community differed, however, among treatments. In addition to shifts in the relative abundance of taxa of the *D. galeata x cucullata* species complex (see further), *D. magna* appeared in the control enclosures during the second half of the experiment.

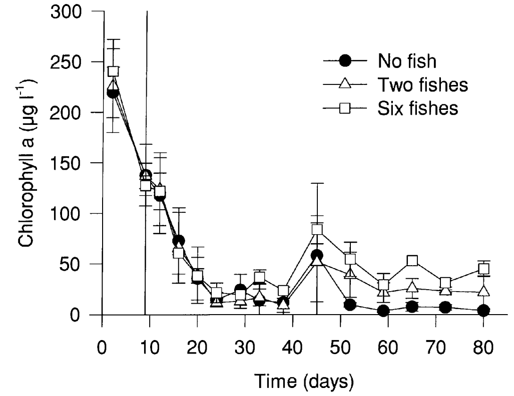

Figure 2. Changes in total chlorophyll-*a* concentration ($\mu g\ l^{-1}$) with time in the enclosure experiment. Data are averaged over treatments. Error bars equal twice the standard error of the mean. The vertical line indicates the introduction of fish in the MF- and HF-enclosures.

Phytoplankton biomass and water transparency

Phytoplankton biomass (Fig. 2) substantially decreased in all enclosures shortly after the start of the experiment (Time effect: *F*: 6.90; $p < 0.01$). After 28 days, the mean concentration of chlorophyll-*a* had decreased to less than 10% of the initial levels measured on Day 2 in all enclosures (from approximately 230 to 19 $\mu g\ l^{-1}$). Chlorophyll-*a* levels remained low in the control enclosures until the end of the experiment. In the enclosures with fish, however, chlorophyll-*a* levels raised again from Day 40 onwards to considerably higher levels than in the control enclosures (Time × Treatment effect: F: 4.83; $p < 0.01$). The reduction in phytoplankton biomass was associated with a strong increase in Secchi-depth. After Day 28, the bottom of all enclosures was visible. Secchi-depth was, however, substantially reduced in the enclosures with six fishes after Day 40 (average value: 0.65m; range: 0.44–0.83m).

Daphnia *taxon composition and population dynamics before the introduction of fish*

At the start of the experiment (Day 2), *D. parvula* was the most abundant *Daphnia* taxon (Fig. 3). Within the *D. galeata x cucullata* species complex, hybrids tended to be most dominant. Initial relative frequencies of *D. galeata* compared to *D. galeata x cucullata* hybrids ranged between 15 and 55% (Fig. 5). *D. cucullata* was not found in any of the samples. During the period preceding the introduction of fish, a positive population growth could be observed for all *Daphnia* taxa (Fig. 3). The instantaneous rate of population increase *r* of *D. galeata* was higher than of *D. galeata x cucullata* ($p = 0.014$). The relative increase of *D. galeata* densities compared to *D. galeata x cucullata* was due to higher birth rates ($p < 0.001$) rather than to lower death rates ($p > 0.05$).

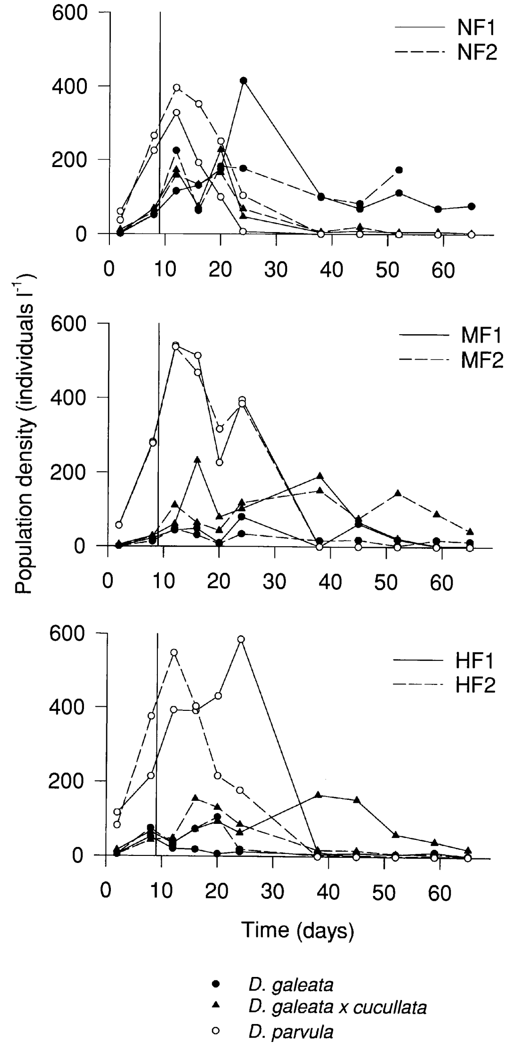

Figure 3. Changes in estimated total population densities (individuals l^{-1}) of *D. galeata*, *D. galeata x cucullata* and *D. parvula* in the enclosures during the experiment. For legend, see Figure 1.

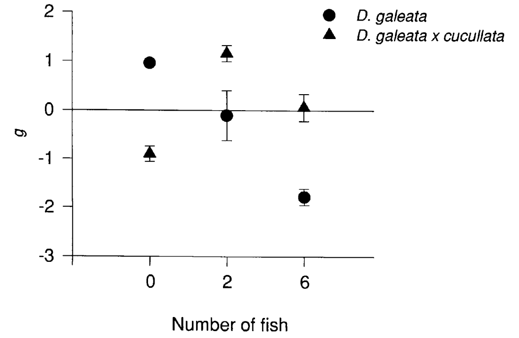

Figure 4. Exponential population growth g of *D. galeata* and *D. galeata x cucullata* in the enclosures, averaged for the period between Day 8 and Day 52. Symbols represent the average per treatment. Error bars equal twice the standard error of the mean.

Daphnia *taxon composition and population dynamics after the introduction of fish*

Despite its high initial population densities, *D. parvula* disappeared from all enclosures after Day 24 (Fig. 3). In the control enclosures without fish, *D. parvula* reached its peak density on Day 12 and then rapidly declined to reach low densities at Day 24. In the enclosures with fish, *D. parvula* tended to reach higher peak densities and remained dominant longer than in the control enclosures, but also decreased to undetectable levels by Day 38. This population decrease of *D. parvula* in the enclosures coincided with about a fourfold reduction of this taxon in the lake (data not shown). *D. galeata* and *D. galeata x cucullata* coexisted in the lake and most of the enclosures throughout the experiment, except in enclosure 'NF2', where both taxa disappeared towards the end of the experiment (Fig. 3). A significant taxon x treatment interaction was found for the g-values of *D. galeata* and *D. galeata x cucullata* (Table 1; Fig. 4). In the enclosures without fish, mean population growth was positive for *D. galeata* and negative for *D. galeata x cucullata*. In the enclosures with two fishes, mean population growth of *D. galeata* approximated zero, while a positive growth could be observed for *D. galeata x cucullata*. In the enclosures with six fishes, *D. galeata* showed a strongly negative population growth, while the abundance of *D. galeata x cucullata* remained unchanged (Fig. 4). As a result, the enclosures without fish became dominated by *D. galeata* during the first 24 days of the experiment, while hybrids became the most abundant taxon in the enclosures inoculated with fish (Fig. 5). Enclosure MF1 formed an exception to this pattern: although enclosure MF1 had been dominated by hybrids for almost one month after the introduction of fish, *D. galeata* progressively became dominant towards the end of the experiment. This was due to a reduction in hybrid abundance rather than to an increase in the abundance of *D. galeata*.

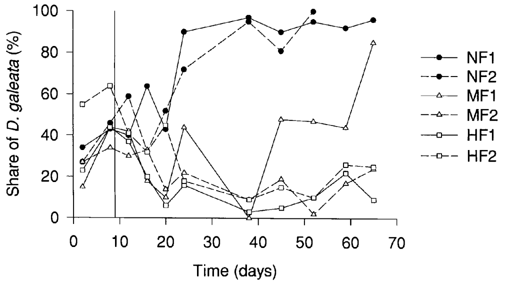

Figure 5. Relative abundance (%) of egg bearing adults of the parental species *D. galeata* in the *D. galeata/cucullata* species complex. For legend, see Figure 1.

During the period preceding Day 24, birth and death rates were different between *D. galeata* and hybrids (Table 2). The birth rates of *D. galeata* were higher than the birth rates of hybrids in all enclosures (Fig. 6). A significant taxon × treatment interaction was found for death rates: in the enclosures with fish, d-values of *D. galeata* were higher than d-values of the hybrids, while in the control enclosures, d-values of *D. galeata* were lower than of the hybrids (Fig. 6; Table 2). As a result, in the control enclosures, the r-values of *D. galeata* were higher than the r-values of the hybrids, both due to higher birth rates and lower death rates. In contrast, r-values of *D. galeata* were lower than r-values of hybrids in the enclosures with fish due to higher death rates, despite higher birth rates. In the period succeeding Day 24, differences in birth and death rates between taxa disappeared and differences in mean r-values between the taxa were non-existent or became in favour of *D. galeata* (Fig. 6; Table 2).

The initial mean adult body length of *D. galeata* was larger than that of the hybrids (Fig. 7). Mean adult body length of both taxa decreased with time and was significantly affected by the presence of fish: adult body length was more reduced in the enclosures with fish than in the enclosures without fish and the effect of the fish treatment increased with time (Table 3; Fig. 7). In addition, a marginally significant taxon by treatment effect was found (Table 3). As high among enclosure variability for mean adult body length potentially masked systematic differences between *D.*

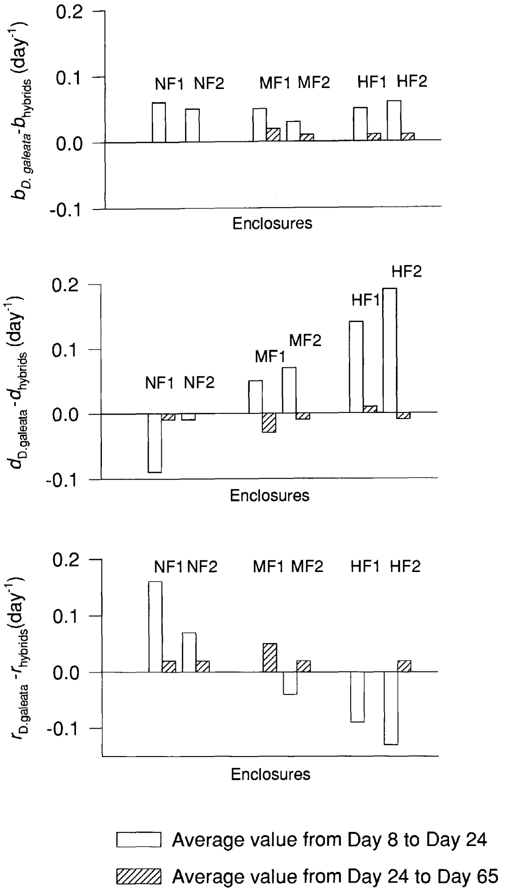

Figure 6. Mean difference in birth rate (*b*), death rate (*d*) and instantaneous rate of population increase (*r*) between *D. galeata* and *D. galeata x cucullata* during the first part (blank bars; Day 8 to Day 24) and during the second part (hatched bars; Day 24 to Day 65) of the experiment. For legend to abbreviations, see Figure 1. Death rates were not calculated for enclosure NF2 during the second part of the experiment, because densities of both taxa dropped below detectable levels.

galeata and hybrids at the within-enclosure level, additional Wilcoxon Matched Pairs Tests were performed for each enclosure separately on paired body length data of consecutive sampling dates (Table 4). For the control enclosures, no significant differences between the taxa were found. In all four enclosures with fish, body length reduction in *D. galeata* was more pronounced than in the hybrid and these differences between the taxa were significant ($p < 0.05$) in three of the four enclosures.

The mean fecundities of *D. galeata* and *D. galeata x cucullata* were dramatically reduced with time and a significant taxon by time interaction was found for *D. galeata* and the hybrid: the fecundity of *D. galeata* was initially higher than the fecundity of the hybrids, but this difference was strongly reduced during the course of the experiment (Fig. 8; Table 3). A significant taxon × treatment effect indicates that the fecundities of the taxa were differentially affected by treatment: compared to the fecundity of the hybrids, the fecundity of *D. galeata* tended to be higher in the enclosures with six fishes than in the control enclosures or in the enclosures with two fishes.

Discussion

Our results provide strong support for the hypothesis that fish predation is important in determining taxon composition in the *D. galeata/cucullata* hybrid complex. The fishless enclosures became soon dominated by *D. galeata*, whereas the enclosures with two or six fishes became dominated by the hybrid *D. galeata x cucullata*, shortly after the introduction of fish. Although the pattern of changes in taxon composition within the *D. galeata x cucullata* complex was more erratic in one of the enclosures with only two fish, our results suggest that the relative fitness of *D. galeata* and *D. galeata x cucullata* hybrids is associated with fish predation. In the enclosures with fish, the reduction in the relative density of *D. galeata* compared to that of the hybrid was due to higher death rates in *D. galeata*. These observations are in agreement with the temporal hybrid superiority hypothesis of Spaak & Hoekstra (1995, 1997) and can be explained a higher vulnerability of the large and more conspicuous *D. galeata* to visual, size selective predation by planktivorous fishes.

Boersma & Vijverberg (1994a) suggested that the relative success of hybrids and parental species might partly be determined by the abundance of food. In life table experiments, these authors compared the performance of hybrids and parental taxa at different food levels. At high food levels (2.5 mg C l^{-1}), hybrids showed a lower development time and a higher intrinsic rate of population increase *r* than the parental species. At low food levels, however, hybrids had a lower development rate, a higher mortality and a lower intrinsic rate of population increase than *D. galeata.* Moreover, threshold values for reproduction were higher in hybrids than in *D. galeata* (Boersma & Vijverberg, 1994a). From these results, Boersma &

Table 1. Results of a two-way Repeated Measures ANOVA, testing for the effects of taxon ('Taxon'), fish number ('Fish') and time ('Time') on the exponential population growth *g* of *D. galeata* and *D. galeata x cucullata*

	g					
Source	Df Effect	df Error	MS Effect	MS Error	*F*	
Taxon	1	6	3.659	0.247	14.84	**
Fish	2	6	13.816	0.247	56.04	***
Time	6	36	7.169	1.165	6.15	***
Taxon × Fish	2	6	27.736	0.247	112.50	***
Taxon × Time	6	36	1.252	1.165	1.07	
Fish × Time	12	36	1.123	1.165	0.96	
Taxon × Fish × Time	12	36	2.827	1.165	2.43	*

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

Table 2. Results of two-way Multivariate ANOVA's, testing for the effect of taxon ('Taxon') and fish number ('Fish') on the birth rates *b* and death rates *d* of *D. galeata* and *D. galeata x cucullata* in the time period between Day 8 and Day 24 and in the time period between Day 24 and Day 59

			b			*d*		
Source	df 1	df 2	Wilkinsons' λ	Rao's R		Wilkinsons' λ	Rao's R	
Day 8 to Day 24								
Taxon	4	3	0.02	39.07	**	0.02	44.56	**
Fish	8	6	0.01	7.17	*	0.00	10.52	**
Taxon × Fish	8	6	0.68	0.16		0.02	4.41	*
Day 24 to Day 59								
Taxon	4	1	0.01	33.56		0.19	1.06	
Fish	8	2	0.00	26.17	*	0.02	1.54	
Taxon × Fish	8	2	0.00	4.24		0.01	2.49	

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

Table 3. Results of two-way Repeated Measures ANOVA's, testing for the effects of taxon ('Taxon'), fish number ('Treatment') and time ('Time') on the mean adult body length and the mean fecundity of the enclosure populations of *D. galeata* and *D. galeata x cucullata*

			Mean adult body length			Mean fecundity		
Source	df Effect	df Error	MS Effect	MS Error	*F*	MS Effect	MS Error	*F*
Taxon	1	4	0.7	0.005	151.21 ***	61.46	0.17	363.45 ***
Fish	2	4	0.325	0.005	70.28 ***	0.91	0.17	5.39 (*)
Time	7	28	0.403	0.004	101.6 ***	181.23	1.08	168.24 ***
Taxon × Fish	2	4	0.022	0.005	4.78 (*)	1.27	0.17	7.52 *
Taxon × Time	7	28	0.003	0.004	0.76	4.43	1.08	4.11 **
Fish × Time	14	28	0.032	0.004	8.19 ***	0.46	1.08	0.43
Taxon × Fish × Time	14	28	0.002	0.004	0.54	0.47	1.08	0.44

(*): $p < 0.1$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

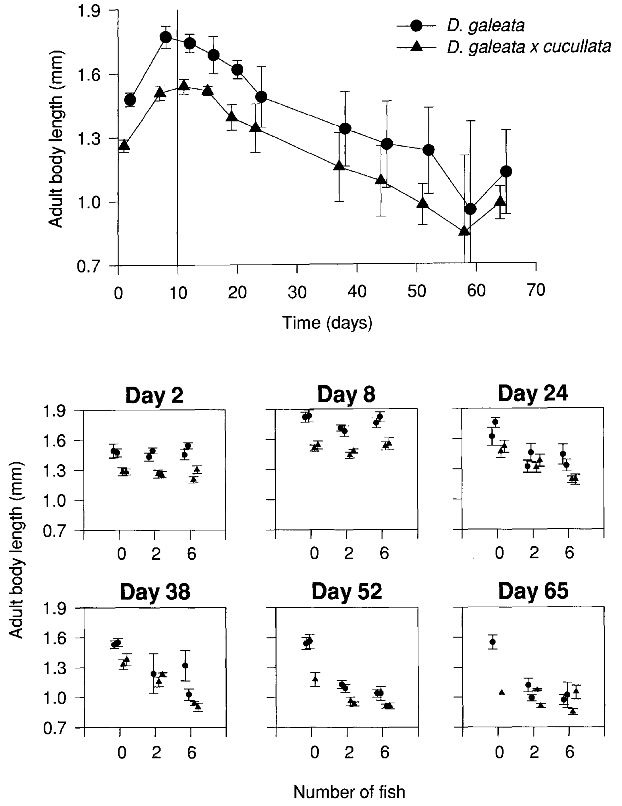

Figure 7. Adult body length of *D. galeata* and *D. galeata x cucullata.* (A) Mean adult body length at different sampling dates averaged over treatments per taxon. Error bars equal twice the standard error of the mean. The vertical line indicates the introduction of fish in the MF- and HF-enclosures; (B) Mean adult body length at different sampling dates as measured for each taxon in each enclosure separately.

Vijverberg (1994a) concluded that hybrids may have a higher performance than *D. galeata* under high food levels, while *D. galeata* may be more successful than hybrids under food limiting conditions. The results of our experiment, however, do not support the idea of hybrid superiority at high food levels in the absence of fish. During the period preceding the introduction of fish, we observed an increased abundance of *D. galeata* relative to *D. galeata x cucullata* in all enclosures. Although chlorophyll-*a* concentrations had dropped from approximately 230 μg l^{-1} at the start of our experiment to levels of about 130 μg l^{-1} at Day 9, phytoplankton biomass and food availability for *Daphnia* remained very high during the entire time interval. Assuming a chlorophyll-*a* to carbon conversion factor of 25 (Weisse et al., 1990), estimated carbon content of the phytoplankton community in the enclosures ranged between 3.25 and 5.75 mg C l^{-}.

It could also be argumented that population losses due to cyclopoid predation may have been higher for the hybrids than for *D. galeata* in our experiment. Indeed, the large cyclopoid copepod *A. robustus* was very abundant during the first time interval of our experiment. Large individuals of cyclopoid copepods can feed on *Daphnia*, and small *Daphnia* individuals are more vulnerable to copepod predation than larger ones (Gliwicz & Umana, 1994). However, during the first time interval of our experiment, mortality rate estimates were not higher for hybrids than for *D. galeata*. Birth rates, on the other hand, were higher in *D. galeata*, and the increase of *D. galeata* relative to *D. galeata x cucullata* was thus due to differences in population growth capacity between the taxa, rather than to differential mortality caused by invertebrate predation.

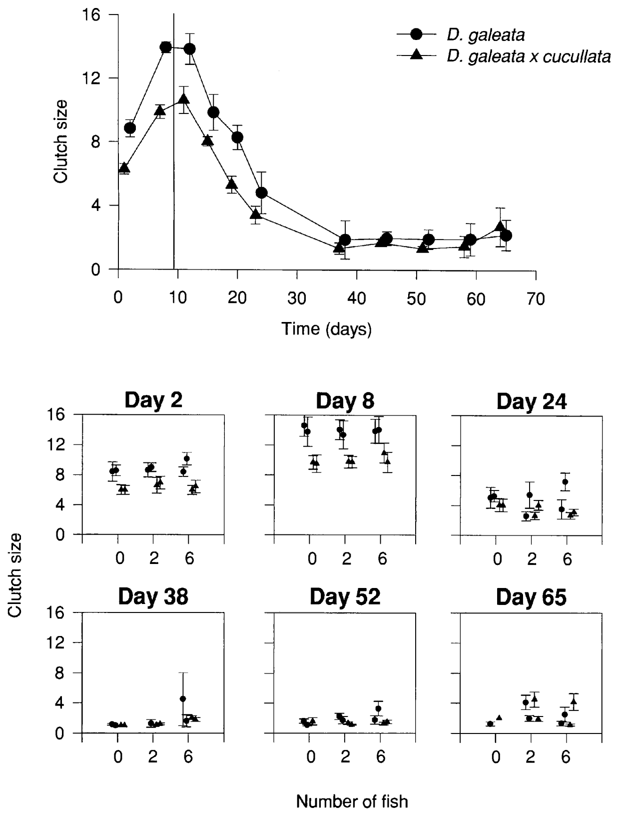

Figure 8. Clutch size of *D. galeata* and *D. galeata x cucullata*. (A) Mean fecundities at different sampling dates averaged over treatments per taxon. Error bars equal twice the standard error of the mean. The vertical line indicates the introduction of fish in the MF- and HF-enclosures; (B) Mean fecundities at different sampling dates as measured for each taxon in each enclosure separately.

Table 4. Results of Wilcoxon Matched Pairs Tests, testing for differences in adult body size reduction between the populations of *D. galeata* and *D. galeata x cucullata*. NF1 and NF2: enclosures with no fish; MF1 and MF2: enclosures with two fishes; HF and HF: enclosures with six fishes.

Enclosure	*T*	*Z*
NF1	11	1.36
NF2	9	1.60
MF1	0	2.67 **
MF2	1	2.55 *
HF1	14	1.01
HF2	0	2.67 **

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$

Although fish predation was found to strongly act to suppress *D. galeata* relative to *D. galeata x cucullata*, our results also show that *D. galeata* populations may continue to coexist with hybrids under moderately high levels of fish predation at least for the time period our experiment lasted (approximately ten weeks). The relative decrease in the abundance of *D. galeata* was strongest shortly after the introduction of fish, and the rates of population growth of both taxa converged during the second part of the experiment. This was due to a reduction in the difference between the death rates of the two taxa. This suggests that *D. galeata* may be relatively more efficient than the hybrid in reducing its (initially higher) vulnerability to fish predation. Several mechanisms might account for such a reduction in vulnerability. First, phenotypic responses of behavioural, morphological or life history traits to the presence of fish kairomone (Boersma et al., 1998; Tollrian & Harvell, 1999; Declerck & Weber, 2002) might, for instance, be more effective in *D. galeata* than in the hybrids. We have no data on behavioural or morphological traits, but we observed strong reductions in adult body size of both *D. galeata* and hybrids after the introduction of fish. In absolute terms, the body size reduction tended to be stronger in *D. galeata* than in the hybrid and this may have lead to a relatively stronger decrease in the vulnerability of this taxon to fish predation. The observation of a stronger reduction of body length in the larger parental taxon *D. galeata* is well in agreement with the findings of Spaak et al. (2000), who investigated the phenotypic responses to fish kairomones of parental and hybrid taxa belonging to the *D. galeata* species complex. Second, along with changes in chlorophyll a-levels, water turbidity was strongly reduced in all enclosures during the first three weeks of our experiment. The increased water transparency undoubtedly intensified visual predation pressure on *Daphnia* in the fish enclosures. In contrast to the control enclosures, however, a strong decrease in Secchi-depth was observed in the fish enclosures approximately one month after the start of the experiment. This increased turbidity coincided with an increased phytoplankton biomass, probably resulting from an enhanced resuspension of sedimented algae and from stimulated phytoplankton growth associated with the effect of fish on the nutrient dynamics (Vanni & Layne, 1997). The increased turbidity may have been especially advantageous for the relatively large and conspicuous *D. galeata*, and may have been an additional factor favouring the persistence of *D. galeata* during the second part of the experiment. Furthermore, *D. galeata* seemed to be better able to exploit the increased amount of phytoplankton in the fish enclosures than the hybrids (e.g. taxon × treatment interaction observed for fecundity).

Although no indications were found for a superiority of hybrids to *D. galeata* at high food levels, the predominance of *D. galeata* to hybrids in the control enclosures supports the conclusions of Boersma & Vijverberg (1994a) that the competitive strength of *D. galeata* is higher than that of *D. galeata x cucullata* under food limiting conditions. In the control enclosures, chlorophyll *a*-levels were strongly reduced during the first weeks of the experiment and subsequently remained low until the end of the experiment. A strong decrease in mean fecundity was observed for *D. galeata* and the hybrids, suggesting severe food limitation. The relative increase of *D. galeata* compared to hybrids was most pronounced between Day 20 and Day 24, the time interval during which chlorophyll-*a* minima were reached.

Given the size selectivity of the fish predation, one would expect that the smallest *Daphnia* taxon *D. parvula* should profit most from the introduction of fish. Although there was an initial tendency towards a stronger population growth of the taxon in the enclosures with fish than in fishless enclosures (e.g. Day 12 to Day 24), this was soon followed by a drastic population reduction. This reduction coincided with about a fourfold reduction in the *D. parvula* lake densities. The reductions of *D. parvula* may have been caused by an unknown environmental factor that affected both the lake and enclosure populations. In contrast to the lake, however, the *D. parvula* populations in the enclosures decreased below detectable levels, and an enclosure effect, such a reduced food levels, cannot be excluded as a potential cause for the seemingly complete disappearance of this taxon in the enclosures.

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Exploitation of a deep-water algal maximum by *Daphnia*: a stable-isotope tracer study

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Abstract

The exploitation of a deep algal maximum by *Daphnia* in the absence of fish predation was studied in large indoor mesocosms. Facing the dilemma of low food but high temperature in the epilimnion vs. high food but low temperature in the hypolimnion, *Daphnia* distribute above and below the thermocline in order to optimise their fitness. Labelling hypolimnetic algae with ^{15}N revealed that the vertical distribution of *Daphnia* is dynamic, i.e., all individuals traverse the thermocline and allocate a certain proportion of their time to feeding in the cold water. The overall energy gain from the deep-water algal maximum is lower than from the same algal concentration in the epilimnion due to the low temperature and the limited time an individual spends in the hypolimnion. The results provide mechanistic support for the hypothesis that *Daphnia* chose their habitat according to an Ideal Free Distribution with Costs model.

Introduction

Recent years have seen increasing interest in deep chlorophyll layers (DCL) in oligotrophic and mesotrophic lakes, and many of theses systems have been described (e.g., Fee, 1976; Padisak et al., 1997; Barbiero & Tuchman, 2001). Deep chlorophyll layers are not only important because of their contribution to primary production (Moll & Stoermer, 1982), they may also be an important food source for zooplankton (Williamson et al., 1996; Adrian et al., 2001). Increased algal densities below the thermocline have been related to nutrient availability (Fee et al., 1977; Fasham et al., 1985) as well as 'pruning' of the upper part of the vertical phytoplankton profile by zooplankton grazing (Richerson et al., 1978) and grazing-related increased vertical particle flux (Sarnelle, 1999). The role of zooplankton is twofold: they may enhance hypolimnetic algal growth by grazing in the epilimnion, which increases light penetration, but they may also feed on the hypolimnetic algal layer and reduce the biomass (Christensen et al., 1995).

Using stable isotopes as tracers in an elegant enclosure study, Pilati & Wurtsbaugh (2003) came to the conclusion that zooplankton grazing is important for the persistence of the DCL. One reason for the persistence of a DCL in the face of high zooplankton densities may be the low temperature, which slows down the grazing rate compared to that in the warm epilimnion. Even more important, however, may be an indirect effect of zooplankton behaviour. If the food concentration is very low in the warm epilimnion but high in the cold hypolimnion, zooplankton is faced with a trade-off. Staying in the epilimnion they will have little energy to produce eggs, but they can develop fast. If they stay in the hypolimnion they can produce many eggs, but development will be slow. They would, therefore, have to find a compromise. In lakes with fish predation, zooplankton migrate into the hypolimnion during daylight to avoid the predator threat (Lampert, 1993). With a DCL but without the temperature effect, it would be profitable for them to stay in the safe hypolimnion during both day and night, but that is not what they do. Williamson et al.

(1996) reported that zooplankton migrated upwards at night despite having much better food conditions in the DCL.

Under low fish predation, zooplankton should no longer migrate in a light-synchronised rhythm, but they should nevertheless spend part of their time in the hypolimnion and the remainder in the epilimnion, which would result in a distribution across the thermocline. Lampert et al. (2003) suggested that filter-feeding *Daphnia* in that case will distribute according to an Ideal Free Distribution with Costs model (Tyler & Gilliam, 1995) in order to optimise their fitness in response to the food and temperature conditions. They found that the proportion of daphniids residing in the hypolimnion to exploit the deep algal layer depended on the temperature gradient according to predictions. The proportion increased when the temperature difference decreased.

The IFD with Costs model assumes that individuals allocate the time spent in the different habitats to optimise their overall fitness. This implies mechanistic hypotheses that can be tested: (1) The observed vertical distribution of daphniids across the thermocline is dynamic. Individuals do not stay where they are observed at a given moment, but move randomly between habitats. The population distribution reflects the average time each individual spends in a particular depth. (2) *Daphnia* gain their food in the cold hypolimnion, but due to the low temperature and the limited time spent there, the energy gain is lower than if the same food concentration would be available in the epilimnion. We tested these hypotheses with a tracer experiment in the same mesocosm system that has been used to by Lampert et al. (2003) to test the distribution model.

Methods

Experimental design and preparations

The experiments were performed in the Plön Plankton Towers, a large indoor mesocosm system consisting of two stainless steel columns, 11.5 m high and approximately 1 m wide. The system has been described in detail by Lampert & Loose (1992). The key features are: (1) complete control over the vertical temperature stratification at 50 cm resolution and the possibility to individually manipulate different portions of the water column, (2) sampling ports with about 50 cm vertical distance to collect profiles of water, seston and zooplankton, (3) near-sunlight light sources above the water column creating an irradiance of 450 μmoles quanta m^{-2} s^{-1}, (4) observation windows at different depths. Each tower holds about 6 m^3 of water.

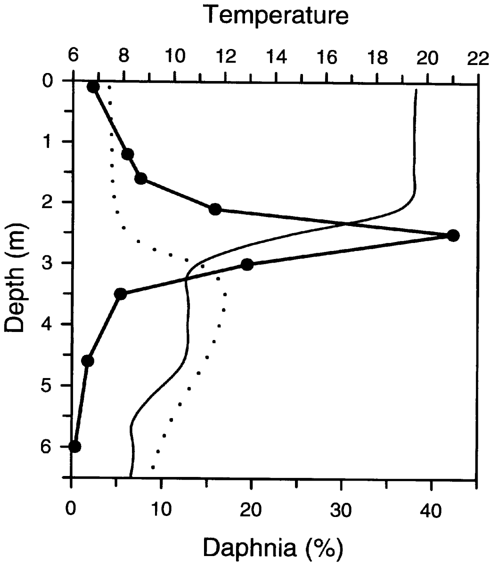

Figure 1. Temperature profile in the mesocosms (solid line) and typical vertical distribution of *Daphnia* (line and symbols) across the thermocline for a hypolimnetic algal maximum (dotted line, relative values) as found by Lampert et al. (2003).

Each experiment started with filling the dry columns with filtered (5–10 μm) water from a nearby mesotrophic lake (Schöhsee). A major thermocline was established at 2.5 m depth with 20 °C above the thermocline (epilimnion) and 10 °C below (hypolimnion). A second, minor thermocline of only 2 °C was established at 5 m depth to isolate the 2.5–5.0 m layer and prevent algae from being mixed downwards (Fig. 1). The light cycle was set to 16 h day and 8 h night. The two towers were treated identically.

Green algae (*Scenedesmus obliquus*) known to be good food for *Daphnia* were mass cultured in 10-l jars in dilute (1:4) Z4 medium (Zehnder & Gorham, 1960). They were added to the columns to make up a final seston concentration of 0.5 mg carbon l^{-1}. Prior to the experiment, mass cultures of a clone of *Daphnia hyalina x galeata* were established in two 100-l containers with *Scenedesmus* as food. When dense populations had been established they were used to inoculate the towers with equal amounts. The daphniids were then allowed to multiply in the towers with

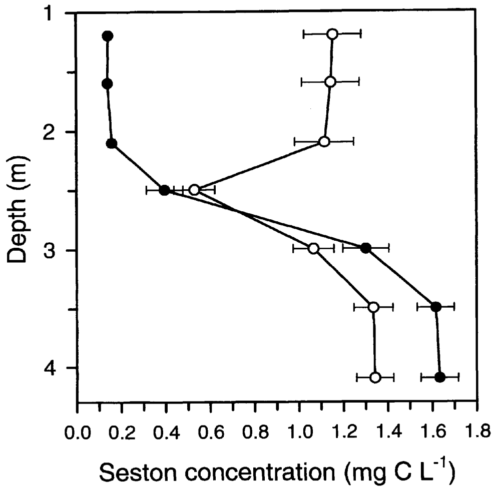

Figure 2. Seston concentration above and below the thermocline in phase 1 (filled circles) and phase 2 (open circles) of the experiment. Means ± 1SE of all dates in two experimental series with two towers each ($n = 16$).

care taken to replenish the grazed food algae. After about 1 week, they had built up dense populations in the towers and consequently the algal populations were grazed down over night. At this point, algae were only added to the layer below the thermocline through a tube. Thus, seston in the epilimnion was quickly reduced to low levels, resulting in a hypolimnetic algal maximum (Fig. 2). Seston concentrations were monitored by withdrawing small water samples from various depths, measuring the total particle volume in a CASY (Schärfe GmbH, Germany) particle counter, and using a calibration curve (volume vs. *Scenedesmus* carbon) to estimate the concentration of particulate carbon. Immediately prior to each experiment, algae were added to the hypolimnion to a level of about 1.5 mg C l^{-1}, which was high enough to prevent algal losses through grazing during successive days without algal replacement.

Feeding experiment

The first experimental series started on 26 November 2001. After the establishment of a deep-water algal maximum, the hypolimnion (2.5–5.0 m) was enriched with ^{15}N-labelled *Scenedesmus* and the distribution of ^{15}N was monitored in seston and daphniids. Before the addition of labelled algae to the hypolimnion (day 0), seston and *Daphnia* were sampled as controls, and then the algal suspension was added through a tube. These algae had been grown in a 2-l culture of *Scenedesmus* in medium with 15% of the inorganic nitrogen replaced by ^{15}N in the form of ^{15}N-(NH$_4$)NO$_3$. The addition of labelled algae had a negligible effect on the total particulate carbon concentration, but enriched the hypolimnetic algae with ^{15}N considerably. Daphniids and seston were collected from the towers daily for the following four days, beginning 24 h after the addition of labelled algae (phase 1). After sampling on day 4, ^{15}N-*Scenedesmus* was also added to the epilimnion, so that algae were now labelled in both layers. Sampling was continued for another four days (phase 2). The towers were then drained, flushed and refilled and the experiment was repeated with the same conditions on 14 December 2001. The two towers and two experimental series resulted in four independent replicates.

Sampling and isotope measurements

Seston and daphniids were sampled through ports at various depths above and below the thermocline and the stable isotope ratios (δ^{15}N) determined. Seston samples were taken from port 4 (at 1.6 m depth) to represent the epilimnion and from port 8 (at 3.5 m depth) to represent the hypolimnion. Volumes of 1.5–2.0 litres of the dilute suspensions and 0.7–1.0 l of the higher concentrations were filtered onto GF/F glass-fibre filters. *Daphnia* were sampled at 5 depths from ports 4 to 8 (1.6, 2.1, 2.5, 3.0, 3.5 m) symmetric to the thermocline. Approximately 50 l of water from each depth per sample were screened through glass traps and returned to the system (cf. Lampert & Loose, 1992). The daphniids were collected on pieces of plankton mesh. Seston and *Daphnia* samples were oven dried at 60 °C over night and stored in a dessicator.

Prior to stable isotope analyses by continuous flow isotope ratio mass spectrometry, *Daphnia* samples were ground to a homogenous powder using an agate pestle and mortar and weighed (0.8–1.0 mg) into tin cups. Small sections were cut from seston filters to determine nitrogen elemental composition. Proportionate sections of the remainder of the filter providing adequate weight of nitrogen for a second round of analyses were then placed into tin cups. Analysis for ^{15}N was carried out using a Carlo Erba NA1500 elemental analyser coupled to a Micromass Isoprime mass spectrometer. Isotope ratios are expressed conventionally using the δ notation in per mil (‰) relative to atmospheric nitrogen. Fish white muscle was used as an internal reference inserted after every five experimental samples. Precision determined from the standard deviation of replicates of the internal reference was ±0.3‰ for δ^{15}N.

Statistics

After testing for assumptions, distributions were compared by repeated measures ANOVA, *t*-test or a non-parametric Kruskal-Wallis one-way ANOVA on ranks. All computations were run with the NCSS statistical package.

Results

The experimental design mimicked a deep-water algal maximum well in all experimental runs (Fig. 2). Seston levels in the epilimnion remained very low and there was no trend to higher seston concentrations during the first four days of the experiment (phase 1). Algae growing in the epilimnion were evidently controlled by *Daphnia* grazing, and seston concentration did not exceed 0.16 mg C l^{-1}. Note that the standard errors plotted in Figure 2 cover both the initial differences between experimental series and the changes over time within a series. Mean seston concentrations in the hypolimnion were lower during the second phase, reflecting algal losses with time. However, seston concentrations still exceeded the incipient limiting concentration for *Daphnia* (approximately 0.4 mg C l^{-1}, Lampert, 1987). A sharp algal minimum occurred at the thermocline after algae had been added to the epilimnion in phase two. This was the result of incomplete mixing in the steep temperature gradient.

Although it was impossible to enrich the hypolimnion with ^{15}N with exactly the same amount in all four replicates, the differences were not large (Fig. 3). A repeated measures ANOVA over the last seven dates detected a significant difference between the means (± SD) for the two experimental series (1443 ± 130 and 1610 ± 154) ($F_{1,14}$ = 13.82, p = 0.002), but there was no significant difference between the towers. ^{15}N-enrichment in the epilimnion increased during phase 1, but reached no more than one third of the hypolimnetic value. After the addition of ^{15}N-labelled algae to the epilimnion (phase 2), the epilimnetic values approached the hypolimnetic values and remained stable. Since there were differences in hypolimnetic ^{15}N signals, all other ^{15}N measurements were related to the respective δ^{15}N of hypolimnetic seston.

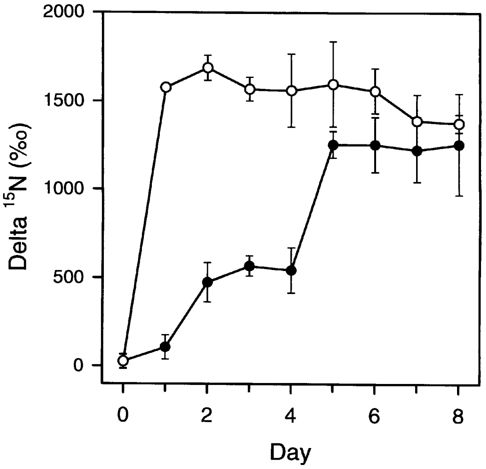

Figure 3. Isotopic enrichment of seston in the epilimnion (full circles) and in the hypolimnion (open circles). Means ± 1SD of two experimental series with two towers each (n = 4).

Daphnia became ^{15}N-enriched over time, but measurements of daphniids sampled at various depths above and below the thermocline showed no difference (Fig. 4). Although there is a slight trend to higher δ^{15}N in *Daphnia* sampled at the thermocline, Kruskal-Wallis one-way ANOVAs on ranks for the individual days found no significant effect of depth. There was no significant difference between towers and experimental series (two-sample *t*-test). Therefore, the five vertical samples of each date and replicate were pooled for further analysis.

Daphniids were significantly enriched in ^{15}N over time (Fig. 5). A repeated measures ANOVA showed a significant effect of time both for the first phase ($F_{1,8}$ = 64.30, p < 0.001) and the second phase ($F_{1,8}$ = 12.12, p < 0.002). During the first phase, the δ^{15}N of *Daphnia* was always higher than that of the epilimnetic seston (Fig. 5), thus the daphniids must have obtained part of their nitrogen from the hypolimnion. After the addition of ^{15}N to the epilimnion in phase 2, *Daphnia* δ^{15}N increased further and approached the value of the surrounding seston.

The dynamics of ^{15}N-enrichment in *Daphnia* resemble two saturation curves approaching different maximum levels in the two phases. Plotting the enrichment data on a logarithmic time scale results in two regression lines (Fig. 6). The regression for the first

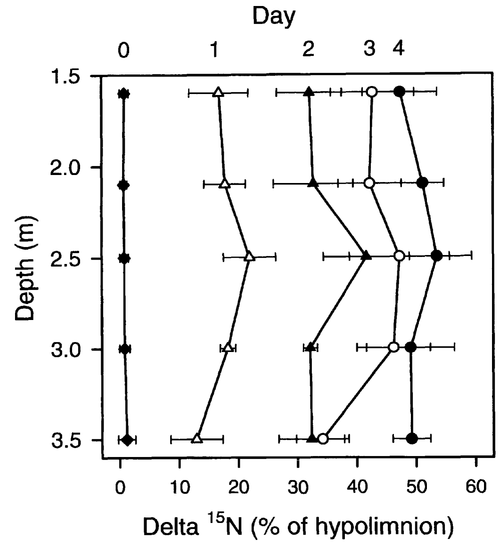

Figure 4. Isotopic enrichment of *Daphnia* sampled at different days at different depths during phase 1 of the experiment. Means ± 1SD of two experimental series with two towers each ($n = 4$). The thermocline is at 2.5 m depth.

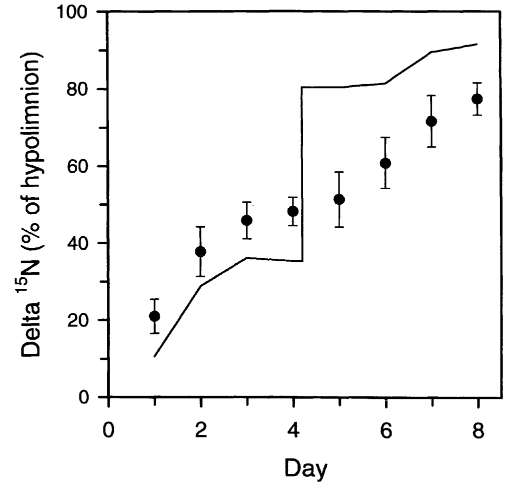

Figure 5. Relative isotopic enrichment of *Daphnia* (filled circles) and epilimnetic seston (solid line) during the two phases of the experiment. *Daphnia*: means ± 1SD ($n = 4$) of pooled vertical samples (cf. Fig. 4). Seston: means for experimental series and towers ($n = 4$). Error bars not shown for clarity (cf. Fig. 3).

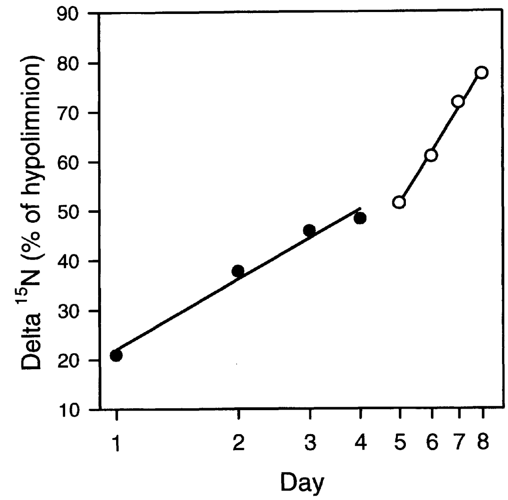

Figure 6. Regressions of relative isotopic enrichment of *Daphnia* on log time for the two experimental phases.

phase is E = 22.4 + 46.6 log$T$ ($r^2 = 0.978$), where E is the relative δ^{15}N as% of the hypolimnetic seston and T is the time in days. The respective regression for the second phase is E = −40 + 131.3 log$T$ ($r^2 = 0.994$).

Discussion

Despite the difficulties of creating identical biological conditions in two towers and two successive experimental series, the replicates developed rather consistently. One major problem is the inability to control the population growth of *Daphnia* in the towers. Despite attempts of equal inoculation, the absolute numbers of *Daphnia* developing differed and this created different grazing pressures. Stable isotope analyses were only possible at the end of the experiments, which resulted in slight differences in ^{15}N additions and seston enrichment between experimental series. Scaling the data to the hypolimnetic δ^{15}N reduced the errors, but the results were not much different from when the analysis was performed with the absolute numbers. We conclude that the results were rather robust.

Although water exchange through the thermocline was strongly inhibited (cf. the minimum in Fig. 2), epilimnetic seston accumulated ^{15}N during the first experimental phase. This enrichment is not simply a consequence of particle transport through the thermocline via eddy diffusion, as the particle density in the epilimnion did not increase. The particle density is a function of algal growth, grazing and vertical transport. Grazing was strong enough to control the particle density in the epilimnion. If algae were transported upward through the thermocline (e.g., during zooplankton sampling) they were removed by grazing immediately. The ^{15}N contained in the algae would then be incorporated by the *Daphnia* and in part be defecated or excreted. Although this pathway cannot be ruled out, the enrichment (one third of the $\delta^{15}N$ in the hypolimnion) is too high to originate from algae entering the epilimnion. It is more likely that daphniids, frequently traversing the thermocline, became ^{15}N-enriched over time, and defecated algal material which they had consumed in the hypolimnion into the epilimnion and in addition excreted ^{15}N-labelled ammonia that could be taken up by the algae. This effect was not so obvious in the enclosure study of Pilati & Wurtsbaugh (2003), but these authors introduced inorganic ^{15}N rather than labelled algae, and only into the metalimnion while the DCL extended much deeper into the hypolimnion.

Epilimnetic enrichment is a first indicator of *Daphnia* movements across the thermocline, but stronger evidence is provided by the uniform enrichment of *Daphnia* sampled at different depths. Had individuals stayed at a preferred depth, those collected below the thermocline would have shown much higher enrichment than those collected above the thermocline. Equal labelling of all groups indicates that on average, they all gathered the same amount of food in the hypolimnion. This is an interesting observation as monitoring of a population *per se* does not provide insight into the behaviour of the individuals (Pearre, 1979). It strongly supports the first hypothesis that the vertical distribution of *Daphnia* is dynamic.

Strong evidence for the second hypothesis is provided by the fact that daphniids exhibited higher ^{15}N-enrichment than epilimnetic seston in phase 1 of the experiment. This is only possible if they gain at least part of their nitrogen in the hypolimnion. Unfortunately, it is not possible to derive quantitative estimates of the share of nitrogen gathered in the DCL. The duration of phase 1 was not sufficient to estimate the $\delta^{15}N$ at equilibrium, and the situation was further complicated by the continuing enrichment of epilimnetic seston. If the ^{15}N-enriched epilimnetic particles were mostly comprised of faecal material, then the food quality was probably lower than the quality of the hypolimnetic particles, which would result in reduced assimilation of ^{15}N and confound the uptake kinetics.

However, the faster enrichment of daphniids in phase 2 of the experiment (revealed by the greater slope of the regression in Fig. 6), shows that DCL conditions limit the energy uptake of *Daphnia*. As predicted in the second hypothesis, daphniids accumulate ^{15}N at a faster rate if food is provided in the epilimnion. This is consistent with measurements of *in situ* grazing rates above and below the thermocline (Lampert & Taylor, 1985) and with observations of W. A. Wurtsbaugh (pers. comm) in a whole-lake tracer study.

This tracer experiment supports the assumptions of the model explaining zooplankton habitat choice as an IFD with Cost (Lampert et al., 2003). Tracer studies may be a useful tool to estimate benefits and costs of zooplankton vertical distribution in response to abiotic (temperature, oxygen) and biotic (food, predation) factors in mesocosms as well as in whole-lake experiments.

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A biogeographical analysis of rotifers of the genus *Trichocerca* Lamarck, 1801 (Trichocercidae, Monogononta, Rotifera), with notes on taxonomy

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Abstract

An analysis of distribution patterns reveals a unique group of Nearctic endemics in *Trichocerca* Lamarck, 1801. This group, comprising 13.4% of all taxa analysed in the genus, is of diverse origin. A glacial origin is postulated for one species. The observed biogeographic pattern of eight others, and possibly two New World taxa, suggests a pre-Pleistocene origin followed by differential extinctions during glaciations in the Nearctic and Palaearctic. In general, endemism in *Trichocerca* is strongly biased towards the Northern hemisphere, with no endemism in tropical regions. This suggests a Laurasian origin of the genus. The analysis further reveals a majority (65.7%) of widely distributed taxa, with strict cosmopolitanism in more than a third of the species analysed. Latitudinal variation is evident in 26.9% of *Trichocerca*, and a warm-water preference appears to be indicated for a majority of these. Although the results should be interpreted with caution due to confused taxonomy, a Southern hemisphere, warm-water and Northern hemisphere, cold water component appear to be present. Comments on the taxonomy and distribution of several species are provided, along with illustrations of poorly known species. Suggestions include elevating *T. maior* (Hauer, 1936) to species rank, and several new cases of synonymy.

Introduction

The study of rotifer chorology, typical of the biogeography of passively dispersing freshwater organisms, has revived during past decades. After a century in which a generalized cosmopolitanism was postulated for such organisms (Jennings, 1900; Rousselet, 1909; Ruttner-Kolisko *in* Dumont, 1980), it is increasingly becoming clear that this hypothesis does not hold for many taxa. The first well-documented cases of rotifer endemism were reported as early as 1940 (Ahlstrom, 1940, 1943). Large-scale analysis of distribution patterns in rotifers revealed latitudinal gradients as well as geographical endemism (e.g., Green, 1972; Pejler, 1977a, b; De Ridder, 1981; Dumont, 1983; Segers, 1996), but it also became clear that much of the apparent cosmopolitanism followed from inadequate taxonomic and faunistic knowledge (Pejler, 1977a; Dumont, 1980, 1983; Koste & Shiel, 1989; Nogrady et al., 1993). These insights largely concur with the results of similar studies in other groups of organisms with passive dispersal strategy (e.g., Anomopoda: Frey, 1986, 1987).

One of the peculiarities of rotifer chorology is, that there are few taxonomic groups in which endemism is focussed in a specific region. The most notable and well-illustrated exception to this trend is the endemic *Notholca* species flock of the Lake Baikal region (Kutikova, 1980; Dumont, 1983). Here, I present a chorological analysis of the genus *Trichocerca* Lamarck, 1801, which appears to exhibit a similarly unique distributional pattern. The genus *Trichocerca* is special amongst Rotifera, as it is ecologically diverse, including freshwater and marine, pelagic, littoral and psammobiotic species. Also, it is the only species-rich genus of Rotifera in which both trophi as well as lorica morphology are taxonomically significant. Surprisingly, there appears to be little concordance in similarity in trophi and lorica morphology. Illustrative of this are several cases of different species having similar external, but different trophi morphology, and *vice-versa*. The fact that both lorica and trophi morphology are varied, and taxonomically significant, makes *Trichocerca* one of the few rotifer genera in which a relatively large number of features is available for analysis (see Ruttner-Kolisko, 1989). On the other hand, it is unfortunate that trophi morphology has not been consistently included in taxonomic treatments, although the significance of this feature in *Trichocerca* had long been realized. In addition, the lorica of *Trichocerca* is asymmetric and easily deformed, leading to the description of poorly contracted or contorted specimens as taxonomic novelties. Study of *Trichocerca* is further hampered by the absence of an early taxonomic revision such as those by H.K. Harring and F.J. Myers, which constitute milestones for the study of taxonomy in many other rotifer groups. As for most Rotifera (e.g., Dumont, 1983; Koste & Shiel, 1989), confused taxonomy impacts on our knowledge on distribution of *Trichocerca*, so published records need to be interpreted with caution. In an attempt to rectify this I provide brief discussions on taxonomy and distribution regarding a number of taxa. Nevertheless, several controversies remain unresolved.

Materials and methods

The material used for the present study is largely based on a revision in progress of the Trichocercidae for the series "Guides to the Identification of the Microinvertebrates of the Continental Waters of the World", edited by H.J. Dumont. The taxonomy follows Koste (1978), with additions and changes as proposed in the recent literature, and including results of original taxonomic research on material from various regions of the world, and from various collections (the Academy of Natural Sciences of Philadelphia, PA, U.S.A.: ANSP; Ghent University, Belgium; the Royal Belgian Institute of Natural Sciences, Brussels, Belgium). Comments on taxonomy, and on a number of species included in the bibliography by De Ridder & Segers (1997) are included. Distributional records are based in part on De Ridder (1986, 1991, 1994) and De Ridder & Segers (1997). For the biogeographical analysis, the conventions of Segers (1996) are followed.

Results and discussion

Taxonomy

A checklist of the taxa considered is presented in Table 1. Two frequently recorded *Trichocerca* species were excluded from the biogeographical analysis because of their confused taxonomy. These are *T. gracilis* (Tessin, 1890) and *T. lophoessa* (Gosse, 1886).The true identity of *T. gracilis* is unclear. A complete and detailed description of this species does not exist, and it appears that several similar taxa have been lumped under this name. Exemplary of the confusion is Myers' (1942) account of the species. Segers & Dumont (1995) depict a specimen with an external morphology matching literature descriptions of *T. gracilis*, but with unique trophi. The case of *T. lophoessa* is similar (e.g., compare the reports on this species by Stemberger, 1979 and Jersabek & Schabetsberger, 1992). These cases illustrate the well-known fact that present-day taxonomy remains an imperfect tool for the study of rotifer biology.

As mentioned above, the present study is largely based on the taxonomic revision by Koste (1978). Some poorly described taxa included in that work are listed as *species inquirendae* in Table 1. In most of these, no account is provided of trophi morphology, and/or the material on which their description is based was in poor condition. Although some may be valid taxa, they are not included in the biogeographical analysis. In the following section, I present arguments for being at variance with the views expressed in Koste's (1978) book.

- **Trichocerca bicristata** (Gosse, 1887) and **Trichocerca mucosa** (Stokes, 1896): Small specimens of *T. bicristata* have frequently been referred to as *T. bicristata* var./f. or subspecies *mucosa*. All verifiable records of *T. bicristata* var./f. *mucosa* concern such specimens. However, *T. mucosa* (Stokes, 1896) is a different, unrelated taxon (Stemberger, 1979; Segers, 1997).
- **Trichocerca braziliensis** (Murray, 1913) and **Trichocerca rattus** (Müller, 1776): It is likely that *T. braziliensis* (= *T. elongata braziliensis* (Murray, 1913) after Koste, 1978; subspecies status rejected by Shiel & Koste, 1992) and *T. rattus* have frequently been confused, as both their trophi and lorica morphology are similar (Segers & De Meester, 1994). I have never found *T. rattus* in collections from regions with a (sub)tropical climate, hence I suspect that many tropical records

Table 1. Checklist of *Trichocerca* species

Trichocerca abilioi Segers, 1993
Trichocerca agnatha Wulfert, 1939
Trichocerca bicristata (Gosse, 1887)
Trichocerca bicuspes (Pell, 1890)
Trichocerca bidens (Lucks, 1912)
Trichocerca brachyura (Gosse, 1851)
Trichocerca braziliensis (Murray, 1913)
Trichocerca capucina (Wierzejski & Zacharias, 1893)
Trichocerca cavia (Gosse, 1886)
Trichocerca chattoni (de Beauchamp, 1907)
Trichocerca collaris (Rousselet, 1896)
Trichocerca cylindrica (Imhof, 1891)
Trichocerca dixonnutalli (Jennings, 1903)
Trichocerca edmondsoni (Myers, 1936)
Trichocerca elongata (Gosse, 1886)
Trichocerca flagellata Hauer, 1937
Trichocerca gracilis (Tessin, 1890)
Trichocerca harveyensis Myers, 1941
Trichocerca hollaerti De Smet, 1990
Trichocerca iernis (Gosse, 1887)
Trichocerca insignis (Herrick, 1885)
Trichocerca insolens (Myers, 1936)
Trichocerca insulana (Hauer, 1937/38)
Trichocerca intermedia (Stenroos, 1898)
Trichocerca kostei Segers, 1993
Trichocerca lata (Jennings, 1894)
Trichocerca longiseta (Schrank, 1802)
Trichocerca longistyla (Olofsson, 1918)
Trichocerca lophoessa (Gosse, 1886)
Trichocerca macera (Gosse, 1886)
Trichocerca maior Hauer, 1936
Trichocerca marina (Daday, 1890)
Trichocerca mollis Edmondson, 1936
Trichocerca mucosa (Stokes, 1896)
Trichocerca multicrinis (Kellicott, 1897)
Trichocerca mus Hauer, 1937/38
Trichocerca musculus (Hauer, 1936)
Trichocerca myersi (Hauer, 1931)
Trichocerca obtusidens (Olofsson, 1918)
Trichocerca orca (Murray, 1913)
Trichocerca ornata Myers, 1934
Trichocerca pediculus Remane, 1949
Trichocerca plaka Myers, 1938
Trichocerca platessa Myers, 1934
Trichocerca porcellus (Gosse, 1851)
Trichocerca pusilla (Jennings, 1903)
Trichocerca pygocera (Wiszniewski, 1932)
Trichocerca rattus (Müller, 1776)
Trichocerca rosea (Stenroos, 189
Trichocerca rotundata Myers, 1937
Trichocerca rousseleti (Voigt, 1902
Trichocerca ruttneri Donner, 1953
Trichocerca scipio (Gosse, 1886)
Trichocerca siamensis Segers & Pholpunthin, 1997
Trichocerca similis (Wierzejski, 1893)
Trichocerca similis f. *grandis* Hauer, 1965
Trichocerca simoneae De Smet, 1990
Trichocerca stylata (Gosse, 1851)
Trichocerca sulcata (Jennings, 1894)
Trichocerca taurocephala (Hauer, 1931)
Trichocerca tenuior (Gosse, 1886)
Trichocerca tigris (Müller, 1786)
Trichocerca uncinata (Voigt, 1902)
Trichocerca vargai Wulfert, 1961
Trichocerca vassilijevae Kutikova & Arov, 1985
Trichocerca vernalis (Hauer, 1936)
Trichocerca voluta (Murray, 1913)
Trichocerca wanarra Segers & Shiel, 2003
Trichocerca weberi (Jennings, 1903)

*List of *species inquirenda*.
Trichocerca taurocephala after Koste & Zhuge, 1996: endemic, Hainan, China (Segers, 1998)
Trichocerca antilopaea (Petr, 1891): unrecognisable; possible synonym of *T. collaris* after Koste (1978).
Trichocerca artmanni (Zelinka, 1927): unrecognisable.
Trichocerca barsica (Varga & Dudich, 1938): unrecognisable.
Trichocerca bicurvirostris (Mola, 1913): unrecognisable.
Trichocerca caspica (Tschugunoff, 1921)(= *T. marina caspica* (Tschugunoff) after Koste, 1978): no account of trophi. Unrecognisable. "Anscheinend mit f. typ. identisch": Koste (1978).
Trichocerca cryptodus (Hauer, 1937): no account of trophi; a relative of *T. cavia* or *T. parvula*?
Trichocerca euodonta (Hauer, 1937): no account of trophi. Unrecognisable.
Trichocerca flava (Voronkov, 1907): not contracted, no account of trophi; unrecognisable.
Trichocerca gillardi Koste, 1978: no account of trophi.
Trichocerca heterodactyla (Tschugunoff, 1921): no account of trophi available; compare with *T. dixonnutalli*.
Trichocerca inermis (Linder, 1904): no account of trophi; compare with *T. dixonnutalli*.
Trichocerca marina longicauda (Tschugunoff, 1921) (= *Rattulus caspicus* var. *longicaudis* Tschugunoff, 1921): no account of trophi. Unrecognisable.
Trichocerca mucripes Ahlstrom, 1938: no account of trophi. North Carolina, U.S.A. Not seen since discovery.
Trichocerca nitida Harring, 1914: no account of trophi.
Trichocerca parva (Manfredi, 1927): unrecognisable.
Trichocerca rectangularis Evens, 1947: close to *T. gracilis* according to Koste (1978). Insufficiently described.
Trichocerca ripli Berzins, 1972: insufficiently described. New Zealand, endemic. Not seen since discovery.
Trichocerca tenuidens (Hauer, 1931): Insufficiently described; compare with *T. tenuior*. Europe, North America.
Trichocerca stenroosi Wulfert after Haberman, 1978: nomen nudum.

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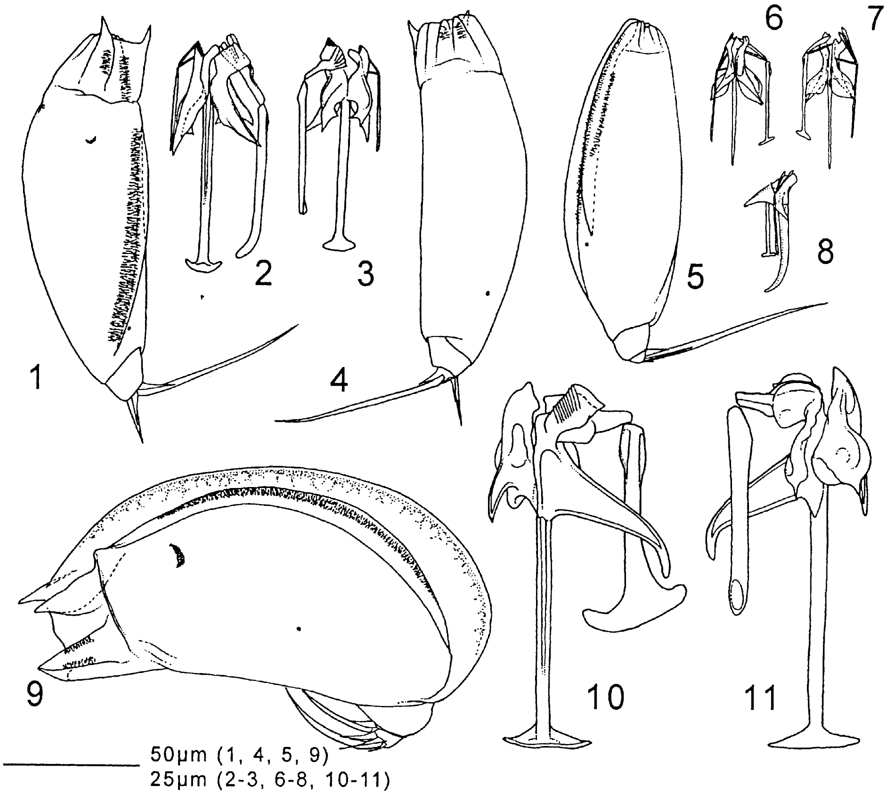
* Species considered valid



Figures 1–4. Trichocerca edmondsoni (Myers). 1: habitus, right; 2: trophi, ventral; 3: trophi, dorsal; 4: habitus, left (1, 3, 4: Pocono Lake, Pennsylvania, U.S.A. 1940: ANSP 602, 2: Id., 1939: ANSP 157, sub. *T. rossae* Edmondson). *Figures 5–8. Trichocerca mollis* Edmondson. 5: habitus, right; 6–8: trophi; 6: ventral, 7: dorsal, 8: right (Minas Gerais, Brazil 1992). *Figures 9–11. Trichocerca maior* Hauer. 9: habitus, left; 10–11: trophi (short, rod-shaped right manubrium not drawn); 10: ventral (left manubrium tilted), 11: dorsal (Pocono Lake, Pennsylvania, U.S.A. 1939. ANSP 689).

of this species concern a different taxon, probably *T. braziliensis*. Of the latter, there are only a few records from temperate regions (e.g., Tasmania: see Shiel & Koste, 1992). *T. rattus* is a variable species, and is here considered to include several variants without taxonomic or geographic significance (e.g., *Trichocerca rattus carinata* (Ehrenberg, 1830), *Trichocerca rattus* f. *globosa* Dartnall & Hollowday, 1985; *Trichocerca rattus minor* Fadeew, 1925).

- **Trichocerca capucina** (Wierzejski & Zacharias, 1893) and **Trichocerca multicrinis** (Kellicott, 1897): Confusion in literature between *T. capucina* and *T. multicrinis* (*T. capucina multicrinis* after Koste, 1978) is suspected, on account of the similar morphology of the two. Whereas *T. multicrinis* has an egg-shaped body, *T. capucina* is slender. Single, unverifiable records of *T. multicrinis* from Siberia (see Koste, 1978), India (Kashmir) and Uganda are not considered here.
- **Trichocerca chattoni** (de Beauchamp, 1907) and **Trichocerca cylindrica** (Imhof, 1891): Tropical records of *T. cylindrica* may refer to the related *T. chattoni* (= *T. cylindrica* var. *chattoni* De Beauchamp; *T. cylindrica chattoni* (De Beauchamp)(sic!) after Koste (1978); subspecies status rejected by Shiel & Koste, 1992). Ecological differences between the two are reported by Shiel & Koste (1992). I have never found *T. cylindrica*

in collections from (sub)tropical regions, nor is *T. chattoni* known to occur in temperate regions.

- **Trichocerca edmondsoni** (Myers, 1936) (**new synonyms**: *T. rossae* Edmondson, 1936, ?*T. compressa* Edmondson, 1937)(Figs 1–4): a comparison of the description and drawings of*T. edmondsoni* (Myers, 1936) and *T. rossae* Edmondson, 1936, and study of specimens identified as *T. rossae* by F.J. Myers (ANSP 157, 602), reveals that both are probably synonyms. In particular, they share an exceptionally stout right toe claw, held at an angle with the longest, left toe claw. Other distinctive features are, the almost symmetrical rami alulae, double frontal mucro, and elongate dorsal keel. Another nominal species with similar morphological features is *T. compressa* Edmondson, 1938, which may also be synonymous. As the name *T. edmondsoni* was included in a publication dated March 20, 1936 (Myers, 1936), and the description of *T. rossae* followed in April 1936 (Edmondson, 1936), the former name is the senior synonym and the valid name for the taxon. The animal is recorded in literature from North America only, however, it also occurs in South America (Brazil: São Paulo, Itirapira; Minas Gerais, Uberaba-Coleto, coll. M. Beatriz Gomes, S. Dabés: H. Segers, unpublished). Edmondson (1938) records his *T. compressa* also from Kashmir, India. This record, the only one from outside the Americas of this species, needs to be confirmed, as the variability of this and related species has long remained unclear (e.g., Myers, 1942), and as similar, and easily confused species exist (e.g., *T. myersi, T. plaka*).
- **Trichocerca elongata** (Gosse, 1886) (**new synonym**: *T. tschadiensis* Pourriot, 1968) (= *T. elongata tschadiensis* (Pourriot, 1968)(sic!) after Koste, 1978), is here interpreted as (a) specimen(s) with particularly strongly contracted head aperture, hence a junior synonym of *T. elongata*.
- **Trichocerca hollaerti** De Smet, 1990 and **Trichocerca lophoessa** (Gosse, 1886): The body of these two species is morphologically very similar. Whereas *T. hollaerti* can only be diagnosed by its trophi, reports on the trophi of *T. lophoessa* are contradictory. Older records of *T. lophoessa* should therefore be interpreted with care. Accordingly, the area of *T. lophoessa* has to be considered insufficiently known, and the identity of *T. lophoessa* f. *carinata* Koste, 1978 cannot be ascertained.
- **Trichocerca insignis** (Herrick, 1885) and **Trichocerca myersi** (Hauer, 1931): *T. insignis* and *T. myersi* are two easily confused, close relatives. The trophi features in the differential diagnosis of the two by Hauer (1931; see Koste, 1978) are not reliable. Both taxa appear to be cosmopolitan, notwithstanding the confusion.
- **Trichocerca insolens** (Myers, 1936) and **T. pygocera** (Wiszniewski, 1932): A synonymy between these two as suggested by Koste (1978) was rejected by Segers (1998). The separation between the rare *T. pygocera* and *T. taurocephala* has been questioned and requires confirmation.
- **Trichocerca insulana** (Hauer, 1937) (**new synonym**: *T. montana* Hauer, 1956): A comparison of the original descriptions of *T. insulana* and *T. montana*, in addition to the study of material from various regions of the world indicates that these two are synonyms. The only reported difference, the straight (*T. insulana*) versus terminally curved (*T. montana*) left manubrium may result from an erroneous observation in the original description of *T. insulana*: it is very easy to overlook the terminal curve in the manubrium in a frontal view as depicted in Hauer's (1937) trophi figure (e.g., compare Figs 10 and 11). The Canadian record of *T. insulana* by Chengalath & Mulamoottil (1975) probably is a misidentification, on account of the different claw length in the specimen depicted.
- **Trichocerca longiseta** (Schrank, 1802) (**new synonym**: *Trichocerca falx* Edmondson, 1936): The specimen depicted by Edmondson (1936) as *T. falx* clearly is a newly hatched individual of *T. longiseta*, in which the spines and toe claws are not yet straightened.
- **Trichocerca longistyla** (Olofsson, 1918): The identity of this species follows its redescription by De Smet (1993). A comparison with *Trichocerca parvula* Carlin, 1939 (nom. nov. for *Diurella parva* Rodewald, 1935 non Manfredi, 1927) reveals a synonymy between the two. A synonymy between *T. longistyla* and *T. rotundata* (sub. *T. parvula*) as suggested by Segers & Sarma (1993) must be ruled out, considering the different trophi of the two taxa. The Brazilian record of *T. longistyla* (sub. *T. rotundata*) by Segers & Sarma (1993) requires confirmation. The single specimen, although clearly related, is much smaller and has trophi that differ slightly from those of North American specimens. Published records of *T. longistyla* (as *T. parvula*) and *T. musculus*

should be interpreted with care, as these two are superficially similar (Koste, 1978).

- **Trichocerca mollis** Edmondson, 1936: Literature records of *T. mollis* are from North America only; unverifiable records from Europe (see De Ridder & Segers, 1997) are not considered here. The animal also occurs in South America (Brazil: Minas Gerais, Uberaba-Coleto, coll. M. Beatriz Gomes, S. Dabés: H. Segers, unpublished). As no account is available on the trophi of the species, some relevant drawings are provided (Figs 5–8).
- **Trichocerca mus** Hauer, 1938: This species had to be considered insufficiently described until the redescription by Nogrady (1983) and Koste (1988). The distribution of this taxon is poorly known, but records appear to indicate that the species is thermophilic.
- **Trichocerca obtusidens** (Olofsson, 1918): This taxon is frequently reported under one of the following names:

  T. minuta (Olofsson, 1918): (junior homonym of *T. minuta* (Gosse, 1886)(ex. *Coelopus*)), and its replacement name *T. arctica* Voigt, 1957). Synonymy suggested by Koste (1978) and De Smet (1988);

  T. relicta (Donner, 1950): synonymy suggested by De Smet (1988), who erroneously gave priority to the junior name *T. relicta*.
- **Trichocerca porcellus** (Gosse, 1851) and *Trichocerca maior* Hauer, 1936 (**new status**) (Figs 9–11): Differences in trophi morphology between *T. porcellus* and *T. porcellus* f. *maior*, as reported by Carlin (1939) are confirmed by personal observations, and appear taxonomically significant. This, in addition to the differences in lorica shape and area of *T. porcellus* and *T. maior*, argue for attributing species rank to *T. maior*.
- **Trichocerca siamensis** Segers & Pholpunthin, 1997 and **Trichocerca uncinata** (Voigt, 1902): *T. siamensis* may have been confused with *T. uncinata*, considering the almost identical external morphology of the two. It is likely that tropical records of the distinctly cold-water *T. uncinata* may refer to *T. siamensis*. There are no verifiable records of *T. uncinata* outside of the Holarctic region.
- **Trichocerca scipio** (Gosse, 1851) (synonym: *T. jenningsi* Voigt, 1957): I here follow Shiel & Koste (1992) rather than Koste (1978) in considering these two taxa synonyms.
- **Trichocerca similis** (Wierzejski, 1893) and **Trichocerca similis** f. **grandis** Hauer, 1965: Although there appear to be no morphological differences between the two forms apart from a difference in body size, they have different areas: whereas *T. similis* is cosmopolitan, *T. similis* f. *grandis* is tropical. The identity of *T. birostris* Minkiewisz, 1900 is unclear. Although *T. birostris* and *T. similis* are reported ecologically different (Carlin, 1943), the absence of a reliable diagnosis (see Koste, 1978) has lead to its synonymy with *T. similis* (see Shiel & Koste, 1992), and prevents its inclusion in the present analysis.
- **Trichocerca tenuior** (Gosse, 1886) (**new synonym:** *T. neeli* Edmondson, 1948): The original description of *T. neeli* by Edmondson (1948) depicts a specimen with heavily contracted head region. All relevant features are strikingly similar to *T. tenuior*, in particular foot and toe spine shape, body, head sheath with single spine, and trophi.*T. tenuior* also frequently inhabits the psammon, similar to what is recorded for *T. neeli.*
- **Trichocerca voluta** (Murray, 1913) (**new synonym**: *T. tropis* Hauer, 1937): A comparison of the original descriptions of *T. voluta* and *T. tropis*, and study of material of the taxon from South America, Africa, and Southeast Asia could not discriminate between the two. They are therefore considered synonyms.
- **Trichocerca taurocephala** after Koste & Zhuge, 1996: The taxon depicted has characteristic trophi which show a striking resemblance to those of *T. mucosa.* The material may represent an unnamed taxon endemic to Hainan, China (Segers, 1998).

Biogeography

A total of 67 taxa (plus *T. taurocephala* after Koste & Zhuge, 1996) are considered in the biogeographical analysis. Of these, one (*T. similis* f. *grandis*) is of infrasubspecific rank, but this position is conditional pending taxonomic revision. The majority of *Trichocerca* (44 or 65.7%, Table 2) have to be considered widely distributed taxa, occurring in both the Eastern and Western hemisphere, without being restricted to the Holarctic region. Of these, true cosmopolitan species are predominant: no latitudinal preference can be distinguished for 26 taxa (38.8%). Due to the difficulty in interpreting records in the group, it is hard to reliably distinguish between different latitudinal groups, however, a cold-water preference can

Table 2. Widely distributed taxa (44–65.7%)

Cosmopolitan taxa (26–38.8%)

T. bicristata (Gosse, 1887)
T. bidens (Lucks, 1912)
T. brachyura (Gosse, 1851)
T. capucina (Wierzejski & Zacharias, 1893)
T. cavia (Gosse, 1886)
T. collaris (Rousselet, 1896)
T. dixonnuttalli (Jennings, 1903)
T. elongata (Gosse, 1886)
T. iernis (Gosse, 1887)
T. insignis (Herrick, 1885)
T. intermedia (Stenroos, 1898)
T. longiseta (Schrank, 1802)
T. marina (Daday, 1890)
T. musculus (Hauer, 1936)
T. myersi (Hauer, 1931)
T. porcellus (Gosse, 1851)
T. pusilla (Jennings, 1903)
T. rosea (Stenroos, 1898
T. scipio (Gosse, 1886)
T. similis (Wierzejski, 1893)
T. stylata (Gosse, 1851)
T. sulcata (Jennings, 1894)
T. tenuior (Gosse, 1886)
T. tigris (Müller, 1786)
T. vernalis (Hauer, 1936)
T. weberi (Jennings, 1903)

Cold-water taxa (5–7.5%)

T. cylindrica (Imhof, 1891)
T. macera (Gosse, 1886)
T. obtusidens (Olofsson, 1918)
T. rattus (Müller, 1776)
T. rousseleti (Voigt, 1902)

Warm-water taxa (6–9.0%)

T. braziliensis (Murray, 1913)
T. chattoni (de Beauchamp, 1907)
T. insulana (Hauer, 1937)
T. flagellata Hauer, 1937
T. ruttneri Donner, 1953
?*T. siamensis* Segers & Pholpunthin, 1997

Pan(sub)tropical taxa (7–10.4%)

T. abilioi Segers & Sarma, 1993
T. hollaerti De Smet, 1990
T. kostei Segers, 1993
T. mus Hauer, 1938
T. simoneae De Smet, 1990
T. similis f. *grandis* Hauer, 1965
T. voluta (Murray, 1913)

be suspected for five taxa, six taxa appear confined to warmer waters. Seven taxa, including *T. similis* f. *grandis*, are Pan(sub)tropical. Patterns of latitudinal distributions were amongst the first biogeographical patterns to be distinguished in Rotifera (Green, 1972), hence it is not surprising to see these revealed in *Trichocerca* as well. The figures appear to indicate a prevalence of warm water preferences for the genus, similar to what is found in *Brachionus* (see Pejler, 1977b; Dumont, 1983) and *Lecane* (Segers, 1996). However, the four Holarctic, and probably also some of the Palaearctic and Nearctic taxa (Table 3) should be considered in this argument, taking into account the preliminary nature of our knowledge on the distribution of *Trichocerca.* For example, *T. obtusidens* is restricted to the Northern hemisphere, and is rather common in cold-water environments, but cannot be considered Holarctic because of records of the species from the Galápagos archipelago (De Smet, 1989). So, a northern-hemisphere component exists in the genus *Trichocerca*, which concurs with *Notholca* and *Keratella.* In contrast to these (e.g., Pejler, 1977b; Dumont, 1983; Battistoni, 1992; De Smet, 2001), no southern-hemisphere cold-water taxa are as yet known in *Trichocerca.* The presence of a warm-water component in *Trichocerca* is at variance with *Notholca*, which is exclusively cold-water.

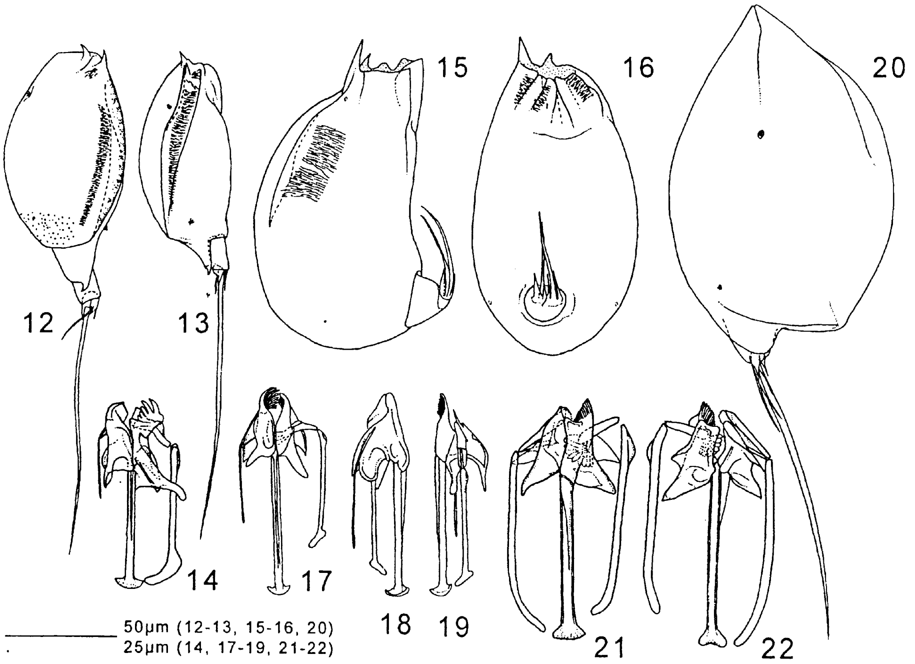
Endemism (Table 3) appears to be rare in *Trichocerca*, and is centred on the Northern hemisphere (Palaearctic, Nearctic, Holarctic taxa). Tropical endemic *Trichocerca* are surprisingly rare. There are no Neotropical, Ethiopian or tropical Australian endem-

Table 3. Endemic taxa: (23–34.3%)

	Holarctic taxa (4–6.0%)	
T. harveyensis Myers, 1941		*T. taurocephala* (Hauer, 1931)
T. maior Hauer, 1936		*T. uncinata* (Voigt, 1902)
	Nearctic taxa (9–13.4%)	
T. bicuspes (Pell, 1890)		*T. ornata* Myers, 1934
T. insolens (Myers, 1936)		*T. plaka* Myers, 1938
T. lata (Jennings, 1894)		*T. platessa* Myers, 1934
T. mucosa (Stokes, 1896)		*T. rotundata* Myers, 1937
T. multicrinis (Kellicott, 1897)		
	New World taxa (2–3.0%)	
T. edmondsoni (Myers, 1936)		*T. mollis* Edmondson, 1936
	Palaearctic (5–7.5%)	
T. longistyla (Olofsson, 1918)		*T. vargai* Wulfert, 1961
T. pediculus Remane, 1949		*T. vassilijevae* Kutikova & Arov, 1985 (endemic to Lake Baikal)
T. pygocera (Wiszniewski, 1932)		
	Old World (2–3.0%)	
T. agnatha Wulfert, 1939		*T. orca* (Murray, 1913)
	Oriental	
(*T. taurocephala* after Koste & Zhuge, 1996)		
	Australia (1–1.5%)	
T. wanarra Segers & Shiel, 2003		

ics; only the enigmatic *T. taurocephala* after Koste & Zhuge (1996) could be an Oriental endemic. This contrasts with results for other groups of rotifer like *Anuraeopsis, Brachionus*, and *Lecane*. Only *Notholca*, a notorious cold-water genus equally lacks tropical endemic representatives (Pejler, 1977b; Dumont, 1983). Basing on the preponderance of endemics in the Northern hemisphere, and notwithstanding the presence of a warm-water, tropical component, a Laurasian origin can be surmised for *Trichocerca*. This concurs with hypothesis on the origin of *Notholca* and, possibly, *Synchaeta* (Dumont, 1983). It should be cautioned, again, that taxonomic tangles and lack of data on distribution weigh heavily on these interpretations. The southwest Australian endemic *T. wanarra* has only recently been diagnosed as separate species in the difficult *T. myersi*-group (Segers & Shiel, 2003), exemplifying that detailed taxonomic studies are a prerequisite to sound biogeographical analysis. The recent record from Thailand of the easily recognized *T. orca*, which had not been seen since its description from New Zealand (see Sanoamuang & Savatenalinton, 2001), shows how little is still known about the distribution of rare species. The case of *T. siamensis*, described only in 1997 but soon after recorded from Brazil and northeast North America (Segers, 1997) is equally revealing.

The North American endemic *Trichocerca* are remarkable. This group contains nine species (13.4% of *Trichocerca*; Table 3), all of which have been found on several occasions. Of these, only one is psammobiotic, thus inhabits a habitat that has only sporadically been investigated worldwide. This stands out against the Palaearctic endemic *Trichocerca*, of which three are psammobiotic (*T. pediculus* – marine, *T. pygocera, T. vassilijevae*). Admittedly, some of the species have been mentioned from localities outside North America, but these concern isolated records, none of which is verifiable by published illustrations or voucher specimens (*T. lata* – Figs 20–22: single records from



. *Figures 12–14. Trichocerca ornata* Myers. 12–13: habitus; 12: left-dorsal; 13: right; 14: trophi, ventral (Atlantic County, New Jersey, U.S.A. 1936: ANSP 283). *Figures 15–19. T. rotundata* Myers. 15–16: habitus; 15: right; 16: ventral; 17–19: trophi; 17: ventral, 18: right; 19: left (Goose Pond, New Jersey, U.S.A. 1996). *Figures 20–22. Trichocerca lata* Myers. 20: habitus, left; 21–22: trophi; 21: ventral; 22: dorsal (Goose Pond, New Jersey, U.S.A. 1996).

Europe (marine!) and New Zealand; *T. ornata* – Figs 12–14: one record from Northeast Asia; *T. plaka*: Europe, New Zealand, but is easily confused with *T. myersi*; *T. rotundata* – Figs 15–19: single record from Afghanistan). These records must therefore be discarded as unreliable. Wang's (1961) Chinese record of *T. bicuspes* is accompanied by a figure, but it is unclear if this is an original drawing. If confirmed, the species would be a rare example of a rotifer with a disjunct Northeast Asia–Northeast North America distribution, similar to *Lecane satyrus* Harring & Myers, 1926 (see Segers, 1995). All nine North American *Trichocerca* occur in the Northeast of North America, only*T. multicrinis* is found as far south as Panama, three reach Florida (*T. bicuspes, T. lata, T. mucosa*: see Ahlstrom, 1934), and one (*T. lata*) is found in Northwest Canada (Chengalath & Koste, 1987). Hence, the North American *Trichocerca* are largely confined to the regions of the Great Lakes and the Northeast. Here, several endemics occur in well-studied groups like *Keratella, Lecane*, and *Notholca* (Stemberger, 1976, 1990a, b; Dumont, 1983; Segers, 1996). That a large number of rotifers belonging to several families are restricted to the Northeast of North America has long been realised. However, this was suspected to be an artefact, considering that the monumental taxonomic works by H.K. Harring and F.J. Myers (e.g., Harring & Myers, 1922, 1924, 1926, 1928; Myers, 1936, 1942; to cite a few) constitute a disproportionate research effort to the rotifers of this region (Segers, 1996). The recent record from Australia of *Dorria*, a monotypic genus long considered endemic to northeast North America, was interpreted in the same way (Shiel, pers. comm.). As H.K. Harring and F.J. Myers did not treat *Trichocerca*, this suspicion can be ruled out and the Nearctic *Trichocerca* may represent the first trustworthy indication that the region really is a centre of endemicity for rotifers.

The northeast North American endemics in the genera *Keratella* and *Notholca* are morphologically very similar to other, more widespread species (Stemberger, 1976, 1990a, b), hence it was hypothesized that they represent recently diverged taxa, probably of glacial origin. On the other hand, a phylogenetic analysis of the endemic *Notholca* of Lake Baikal (Kutikova, 1980) reveals that this group is morphologically distinct, and is even considered consistent with genus rank by Dumont (1983). The origin of the North American *Trichocerca* is less clear, and more diverse. There is no doubt that *T. multicrinis* is a close relative of *T. capucina,* and a glacial origin of this species, similar to the above-mentioned Brachionidae, can be surmised. In contrast to the endemic *Keratella* and *Notholca* species, however, its range encompasses North and Central America. The restricted distribution of the northeast North American *Keratella* and *Notholca* was attributed to habitat characteristics, and/or the inability to produce resting eggs as propagules (Dumont, 1983; Stemberger, 1990; Segers, 1996). This may hold for a number of *Trichocerca,* but not for *T. multicrinis,* considering the vast range of this pelagic species.

In contrast to *T. multicrinis,* the relations of the other species are less obvious.*T. bicuspes* probably belongs to the *T. rattus*-group, by its similar trophi (see Nogrady, 1989), *T. plaka* is close to *T. myersi* (see Segers & Shiel, 2003), *T. mucosa* has trophi similar only to *T. taurocephala* after Koste & Zhuge (1996). The relations of the other species in the group, and those of the two New World endemics (*T. mollis* and *T. edmondsoni*), cannot be ascertained at the moment. However, regardless of their precise phylogenetic relations, it is clear that they represent independent evolutionary lineages. Also, the large morphological dissimilarity with their closest relatives indicates that they must be the product of relatively ancient, probably pre-Pleistocene, radiations. As such, they may be relicts of an endemic Nearctic fauna. The *Trichocerca* species now restricted to the northeast of North America may have survived the glaciations in local refugia, those which occur as far south as Florida (or to South America in the case of *T. mollis* and *T. edmondsoni*) may have done so by migrating along with the shifting climate. The second option is much less likely in the Palaearctic, due to the major mountain ranges having an East–West orientation, hence becoming effective barriers against North–South migration of organisms during glaciations. So, that relatively fewer Palaearctic than Nearctic taxa survived the Pleistocene glaciations is consistent with contemporary theories on the impact of glacial extinctions (see, for example, Brown & Lomolino, 1998). It is unfortunate that so little is known about the *Trichocerca* of Beringia, as a particularly interesting fauna can be expected there, based on the present interpretations.

Conclusions

Analysing distribution patterns in 67 taxa of *Trichocerca* reveals a majority (65.7%) of widely distributed species, with strict cosmopolitanism in more than a third of the taxa. Latitudinal variation is evident in 26.9% of *Trichocerca*, and a preference for warm waters appears to be indicated. Endemism, on the other hand, is strongly biased towards the Northern hemisphere, with no endemism in the Neotropical, Ethiopian, and (?)Oriental regions, and tropical Australia. Moreover, a distinct Southern-hemisphere temperate *Trichocerca* fauna cannot be identified. These results appears to indicate a Laurasian origin of the genus, although both a Southern hemisphere, warm water and Northern hemisphere, cold-water component can at present be distinguished in the genus.

It should be cautioned that the preponderance of widely distributed taxa and the low degree of endemicity might at least partly result from the inability of present-day taxonomy to distinguish between closely related species. However, and notwithstanding the confused taxonomy, a group of Nearctic endemics stands out in *Trichocerca*. The origin of the taxa in this group is diverse: *T. multicrinis* is a close relative of *T. capucina* and may be of glacial origin, the others and two New World taxa are morphologically distinct, to the extend that they probably belong to independent lineages or, at least, are the result of more ancient radiations. A pre-Pleistocene radiation of a Northern hemisphere fauna, followed by differential extinction during the glaciations in the Nearctic and Palaearctic is postulated to account for the relatively high degree of endemicity of *Trichocerca* in the Nearctic.

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On a remarkable South African giant ostracod (Crustacea, Ostracoda, Cyprididae) from temporary pools, with additional appendages

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Abstract

Liocypris grandis, a Red Data List species, presumed extinct, has been found for the first time since its original description in 1924 in the Western Cape Province in South Africa, in an area about 200 km NE from its type locality. The species is redescribed. Apart from the seven paired appendages, normal for the Podocopida, five additional paired appendages also occur. As these structures do not occur in the (A-1) female juvenile stage, the significance of these structures is deemed to be reproductive. Owing to this association with the (female) genital organs, these structures are here termed R1–R5. Their putative homology is discussed; it is deduced that, contrary to earlier claims, the (female) genital organs in ostracods might be homologous to more than one (thoracic) limb, in this case 3–5 limbs and segments. The structures are either non-functional relicts, or are functional with regard to brood care or mating behaviour. The phylogenetic signal delivered by the presence of these structures is highly obscure; they could be from most ancestral to highly derived, even independent of the phylogenetic position of *Liocypris* itself. The presence of these R-appendages, together with some other features, provokes the erection of *Liocypridinae* subfam.n. within the family Cyprididae.

Abbreviations: A1 – Antennula; A2 – Antenna; Cp – carapace; CR – Caudal Ramus; H – height of valves; L – length of valves; ls – lateral shield of hemipenis; LV – left valve; Md – Mandibula; ms – medial shield of hemipenis; Mx1 – Maxillula; R – Rome organ; R1–R5 – additional appendages in adult females; RV – right valve; St – Sternum II; T1 – first thoracopod; T2 – second thoracopod; T3 – third thoracopod; – Chaetotaxy of the limbs follows the model proposed by Broodbakker & Danielopol (1982), revised for the A2 by Martens (1987). Higher taxonomy of the Ostracoda follows the new synopsis by Horne et al. (2002).

Introduction

Ostracods are small (typical length is 1 mm), bivalved Crustacea which abound in all kinds of marine and non-marine, even terrestrial, environments. Ostracoda are generally divided into two subclasses, Myodocopa and Podocopa (Horne et al., 2002). The former are entirely marine, the latter have both marine and non-marine groups. Within the Podocopa, the order Podocopida has 5 suborders, of which the Cypridocopina have the most speciose non-marine lineages. The family Cyprididae within this group can comprise up to 80% of all non-marine species in certain, mostly tropical, areas (Martens, 1998).

Ostracods are unusually conservative with regard to the number and form of their appendages. Whereas valve size, structure and shape can vary widely amongst even closely related groups, most (all?) podocopid ostracods have 7 pairs of appendages (A1, A2, Md, Mx1 and three pairs of thoracopods), a pair of copulatory organs and a pair of caudal structures, previously named furca, now referred to as caudal rami (or uropods, Meisch, 2000), because of unclear homologies with other Crustacea. There is thus a reduction in total number of appendages. For example, the Mx2 seems to be completely missing, although the brush-shaped organ in some male cytherids might be a remnant of this limb, and no abdominal appendages at all are known in podocopid ostracods. Also, the morphology of most appendages is conservative, with the exception of the fifth limb (first thoracopod), which can be either a walking limb (e.g. Cytheroidea, Darwinuloidea), a weakly-developed palp (female Cyprididae) or a two-segmented prehensile palp, often asymmetrical (male Cyprididae). The third thoracopod is either a walking leg (Bairdioidea, Sigillioidea, Cytheroidea, Darwinuloidea) or a cleaning leg (Cyprididae). But all other appendages are highly similar in the podocopid groups. This limited number of appendages, as well as their conservative morphology, have been ascribed to physical constraints faced by bivalved animals where the carapace encloses the entire animal. But this is certainly not true for the Spinicaudata (up to 32 pairs of trunk limbs) or the Laevicaudata (12 pairs in females, 10 in males) (Fryer, 1987).

The paired copulatory appendages in ostracods can be quite large, especially in males (Cohen & Morin, 1990). The hemipenes can comprise up to one third of the total body (Martens, 1990 on *Limnocythere*), the female reproductive organs are generally much smaller. The homology of these paired reproductive organs is a long-standing problem, and little information is available.

The recent rediscovery of the rare, giant ostracod *Liocypris grandis* in temporary pools in the Western Cape Province allowed a thorough redescription of this enigmatic animal. Adult females appeared to have additional paired appendages, associated with the copulatory organs. These are here described; their putative homology is discussed. Because of the presence of these features, the species and genus are transferred to a new subfamily within the Cyprididae, Liocypridinae n.subfam.

Taxonomic descriptions

Class Ostracoda Latreille, 1806
Subclass Podocopa G.W. Müller, 1894
Order Podocopida Sars, 1866
Suborder Cypridocopina Baird, 1845
Superfamily Cypridoidea Baird, 1845
Family Cyprididae Baird, 1845

Liocypridinae subfam. n.

Diagnosis:
Giant Cyprididae (L >= 5 mm), with arched, laterally compressed carapaces and wide anterior calcified inner lamella. Non-reproductive appendages typical of the family. A1 without flagellated claws. Muller-organ present between A1 and A2. A2 in males with both claws G3 and G1 short and claw-like. Mx1 with smooth *Zahnborsten* on third endite, terminal palp segment rectangular. Md with alpha-seta with broad base and flagellum-like tip, beta-seta stout and hirsute, gamma-seta stout and hirsute in the terminal half. T1-palp in females long and stout, three terminal setae relatively short; 'c' seta absent; male prehensile palps asymmetrical, right palp larger than left palp. T2 a walking leg, with penultimate segment divided. T3 a slender cleaning leg, apically with a pincer. Caudal rami symmetrical, slender, attachment without Triebel's loop. Adult females with a series of additional paired appendages associated with the genital operculum. Hemipenis large, lateral shield consisting of 3 lobes; internal anatomy simple.

Remarks: This new subfamily of giant ostracods is distinguished from all other subfamilies in the Cyprididae, giant or other, by the presence of the additional appendages in the adult female. Additional features are the aberrant shape of the valves and the unusual distal chaetotaxy of the A2 with both G1 and G3 short and claw-like (G3 is a seta and G1 a slender claw in Megalocypridinae and Cypridinae). It can also be distinguished from other subfamilies by the absence of various features, i.e. from the Cypricercinae by the absence of the Triebel's loop in the attachment of the caudal rami, from the Megalocypridinae by the absence of flagellated claws on the A1, from the Eucypridinae by the absence of the 'c' seta on the T1, etc. The most similar-looking giant Cyprididae are the species of the genus *Amphicypris* Sars, 1901 (?syn.: *Cypriconcha* Sars, 1926), but these lack the additional female appendages present in the Liocypridinae n.subfam. In addition, the sexual dimorphism in valve shape is pronounced and rather unusual for Cyprididae, being more typical of the Candonidae. Nevertheless, because of the structure of the appendages (natatory setae and chaetotaxy of the A2, T3 being a cleaning limb, etc.) the present lineage remains in the Cyprididae.

Liocypris Sars, 1924

Type species:
Liocypris grandis Sars, 1924 (by original designation)

Diagnosis (amended from Sars, 1924):
Valves large, laterally flattened, in lateral view highly arched in the posterior half of the valves; external valve surface smooth, calcified inner lamella anteriorly wide, posteriorly narrow. All appendages elongated. A1 with long, A2 with short natatory setae. Mx1-palp segment rectangular, c. 1.5 times as long as the basal width. T1 in females with unusually elongated palp. T2 with seta d1 c. 3 times as long as d2. Caudal ramus elongate, with 2 claws and 2 setae, all apically or subapically inserted; attachment slender and without additional branches or loops. Hemipenis large, ls with three large lobes, ms rounded. Females with 5 additional pairs of appendages associated with the genital organs, i.e.: an anterior plate-like expansion, followed by a worm-like structure, medially with a palp- and respiratory plate like structure, caudally with a distally pointed, pseudo-segmented rod and a large, hook-like structure, the latter resembling a giant limb-*Anlage*.

Remarks:
As the new subfamily is monogeneric, it is difficult to determine which features are relevant at the level of the genus. If a second genus in this subfamily were to be discovered, it is possible that several features listed above might be shifted between taxonomic levels. The present diagnosis is amended from the original one (Sars, 1924) in a number of places, i.e. the morphology of the valves, the d-setae on T2, and especially the additional appendages in the female.

Liocypris grandis Sars, 1924
(Figs 2–7)

Type locality:
Stompneus, c. 100 km N of Cape Town (approximate coordinates: 32° 48′ S, 17° 55′ E).

Type material
Type material was collected by W.H. Purcell, and is presently curated in the South African Museum (Cape Town, RSA) and the Zoological Museum (Oslo, Norway).
Lectotype (here designated): a dissected male, with soft parts in glycerine in a sealed slide, valves stored dry in a micropalaeontological slide (SAM A6281) (dissection: P. De Deckker).

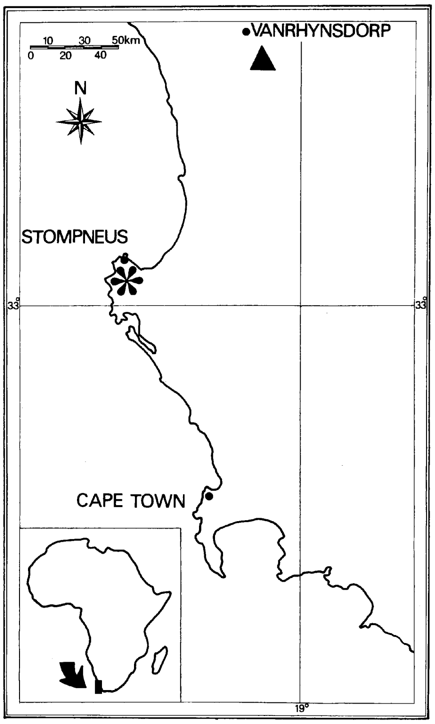

Figure 1. Localities of *Liocypris grandis* Sars in the Western Cape Province of South Africa. Asterisk = type locality near Stompneus; triangle = new localities near Van Rhynsdorp.

Paralectotypes:

- A male, with soft parts dissected in glycerine, valves lost (no nr, dissection: K. G. McKenzie, 31.8.1978)
- A female, with soft parts dissected in glycerine in three separate slides, valves stored dry in a micropalaeontological slide (dissection: K. Martens, km.1112a–d).
- A female, with soft parts *in toto* glycerine in a sealed slide, valves stored dry in a micropalaeontological slide (dissection: K. Martens, km.1217a,b).
- SAM A11309: a tube with 2 adult females (1 with LV missing), 3 juvenile females (A-2?) and several fragments of valves of various stages.
- One slide in the Oslo Museum (nr 11148), labelled as *Liocypris magna*, most likely constitutes the specimen investigated by Sars.

Other material used
1. Blinkvlei, near Van Rhynsdorp (Cape Province, South Africa). Coordinates: 31° 44′ 21″ S, 18° 55′

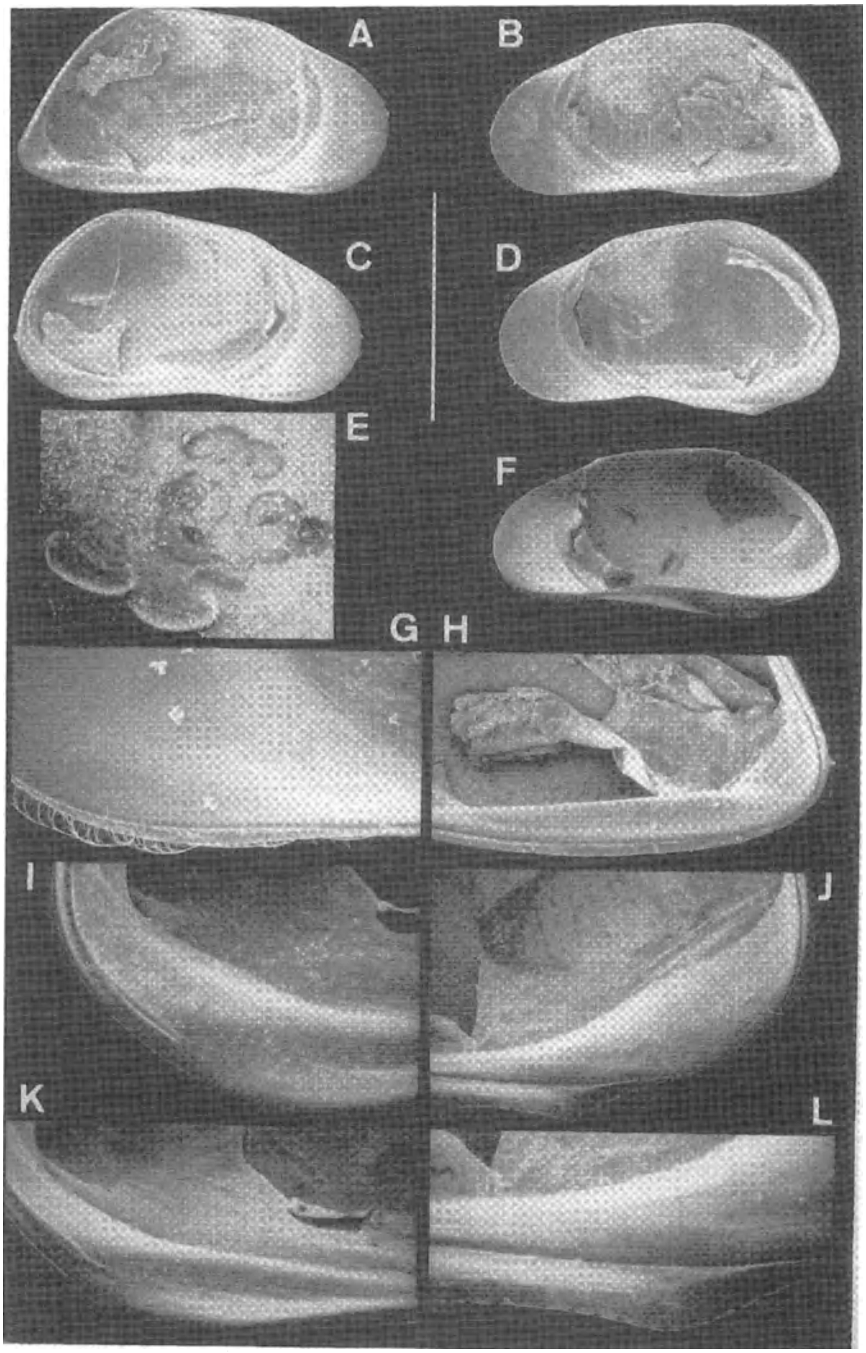

Figure 2. Liocypris grandis Sars. (A) ♀, RV, internal view (KM. 3242). (B) ♀, LV, internal view (idem). (C) ♂, RV, internal view (KM.3243). (D) ♂, LV, internal view (idem). (E) ♂, RV, internal view, detail of central muscle scars (KM.3112). (F) ♂, RV, internal view, tilted to show extent of ventral selvage (idem). (G) ♀, RV, internal view, detail of antero-ventral margin (KM.3113). (H) Idem, detail of postero-ventral margin. (I) ♂, LV, internal view, detail of posteroventral margin (KM.3112). (J) ♂, RV, internal view, detail of posteroventral view (idem.). (K) ♂, LV, internal view, detail of posteroventral margin (idem). (L) ♂, RV, internal view, detail of ventral view (idem.). Scale (in μm) = 3125 for A–D, F; 1316 for J,H; 909 for I,K; 667 for G; 556 for E,L.

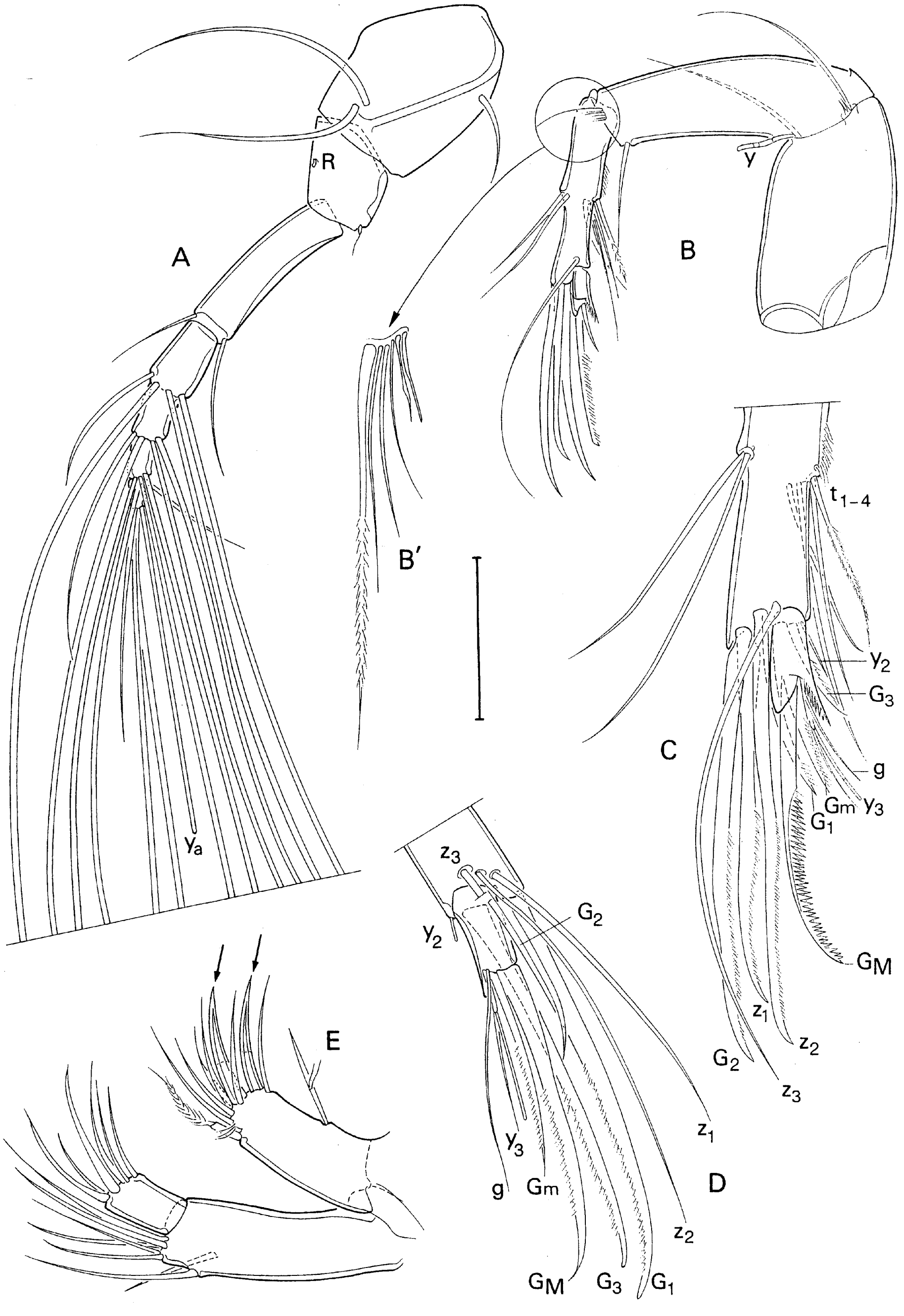

Figure 3. Liocypris grandis Sars. (A) ♂, A1, (KM.3246). (B) ♂, A2, (KM.3246). B'. A2, detail of natatory setae (idem). (C) A2, detail of distal chaetotaxy (idem). (D) ♀, A2, detail of distal chaetotaxy, (KM.3245). (E) ♂, Mx1, detail of palp and third endite, (KM.3246). Scale (in µm) = 435 for A,B; 217 for C–E, 108 for B'.

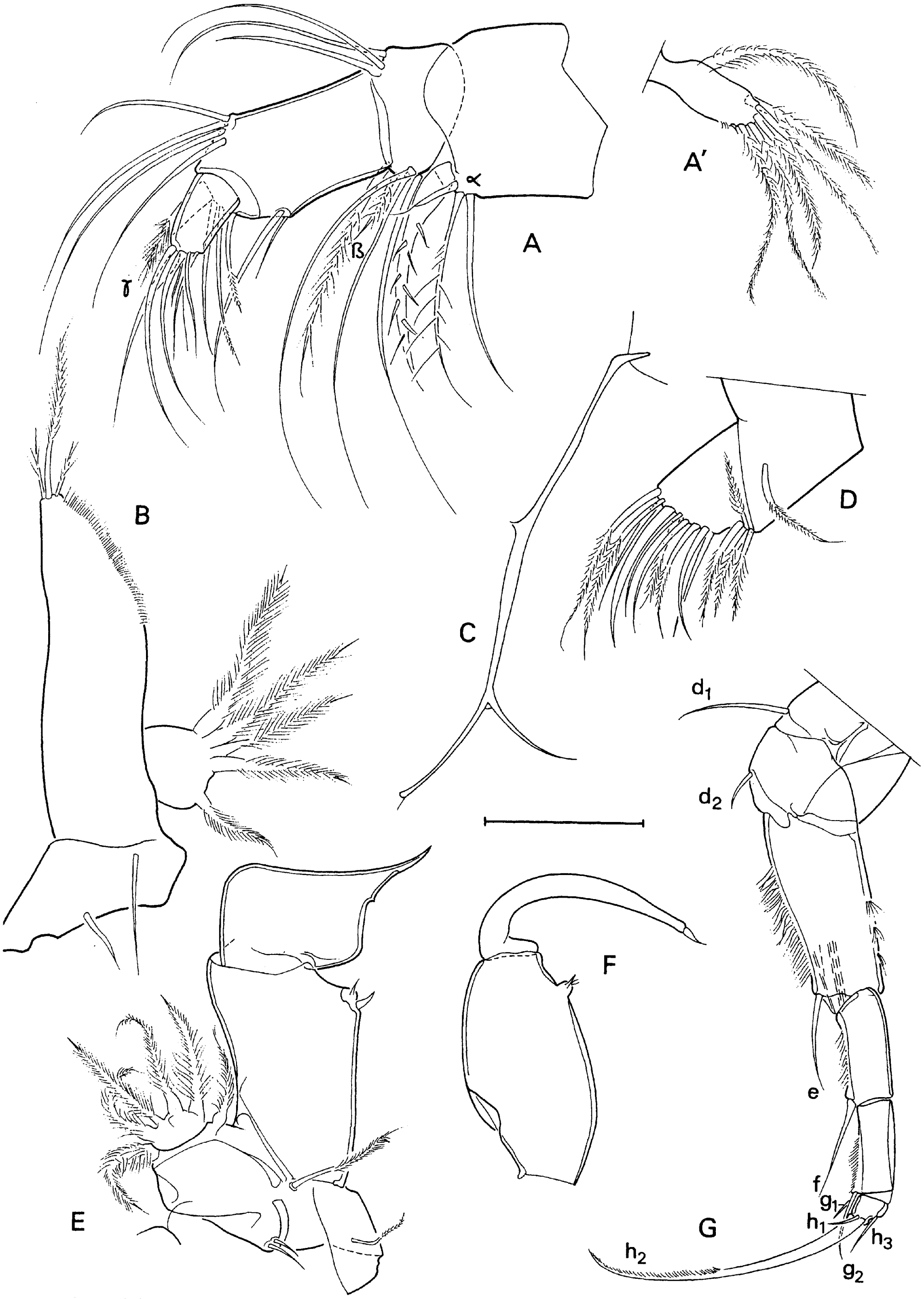

Figure 4. Liocypris grandis Sars. (A) ♂, Md-palp, (KM.3246). A′. Md-palp, detail of respiratory plate (idem). (B) ♀, T1, palp and respiratory plate, (KM.3245). (C) ♂, attachment of caudal ramus, (KM.3246). (D) ♂, T1, detail of chaetotaxy of basipodite, (KM.3246). (E) ♂, T1, right prehensile palp and respiratory plate, (KM.3246). (F) T1, left prehensile palp, idem. (G) ♂, T2 (KM.3246). Scale (in μm) = 435 for A',B,C,E–G; 217 for A,D.

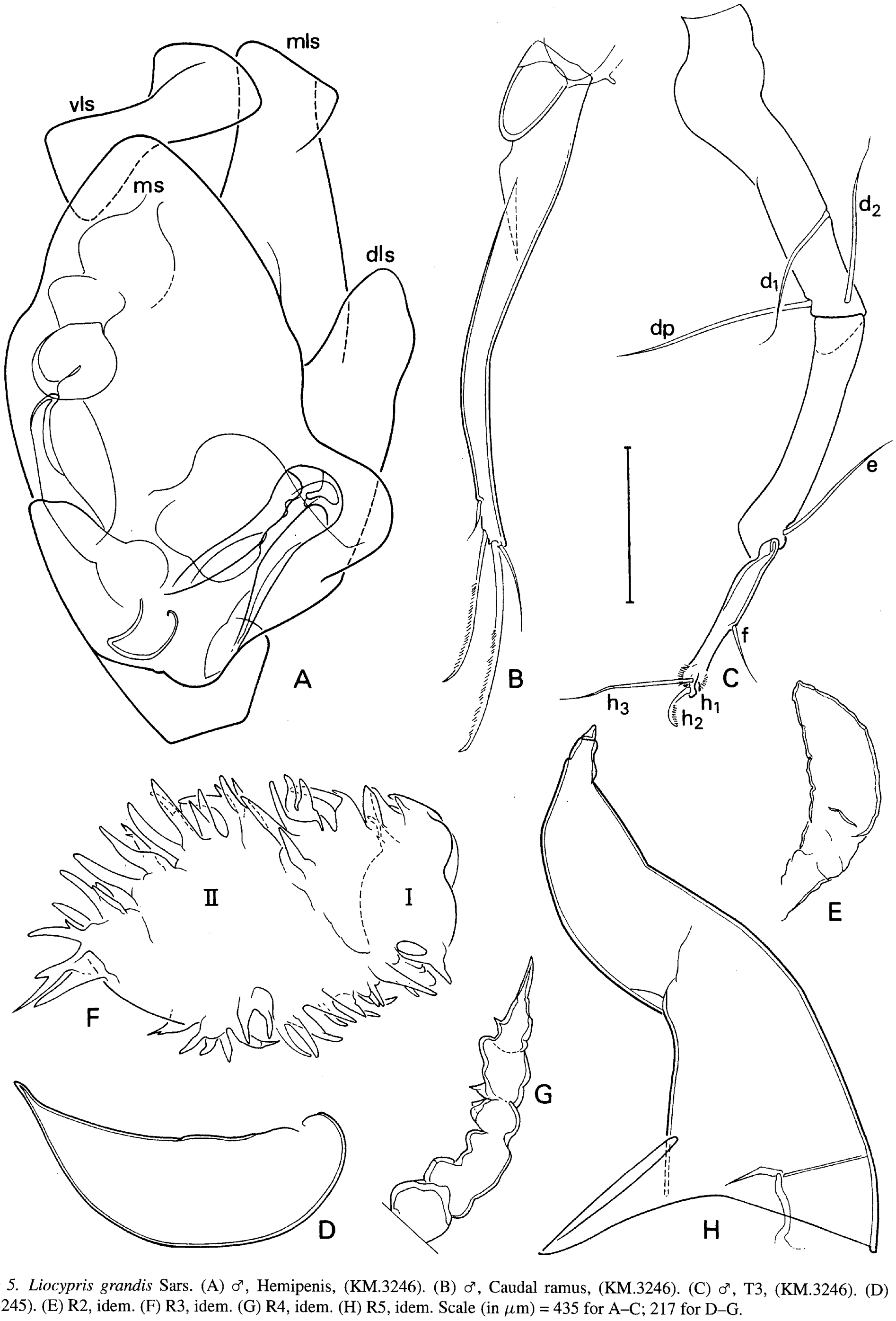

Figure 5. Liocypris grandis Sars. (A) ♂, Hemipenis, (KM.3246). (B) ♂, Caudal ramus, (KM.3246). (C) ♂, T3, (KM.3246). (D) ♀, R1, (KM.3245). (E) R2, idem. (F) R3, idem. (G) R4, idem. (H) R5, idem. Scale (in μm) = 435 for A–C; 217 for D–G.

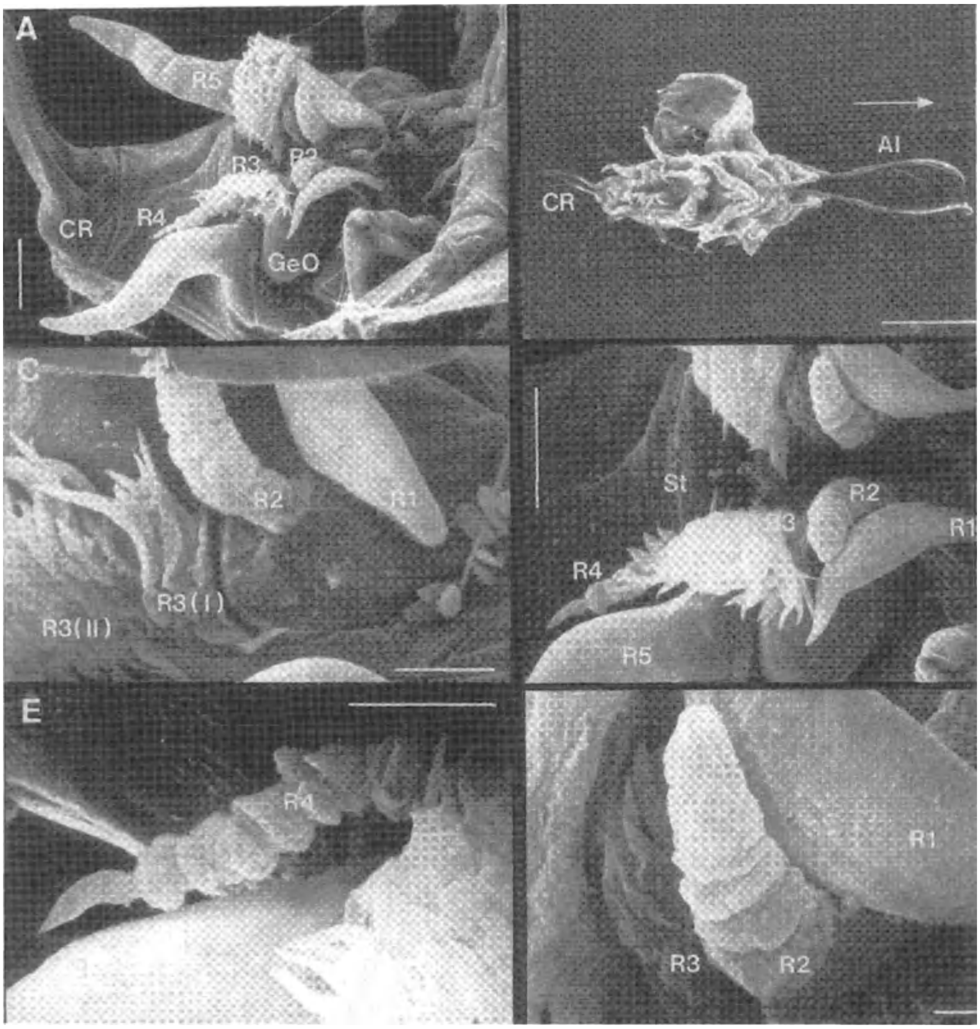

Figure 6. Liocypris grandis Sars. (A) Ventral view of caudal part of adult female, showing paired appendages R1–R5. (B) Ventral view of complete adult female. (C) Idem, detail of R1, R2 and palp of R3. (D) Idem, detail of R1–R4. (E) Idem, detail of R4. (F) Idem, detail of R2 and part of R1. Scales (in μm): A = 200; B = 2000; C = 100; D = 200; E = 100; F = 50.

23″ E. Blinkvlei is a large, circular (diameter c. 250 m) temporary pan, rather shallow (mostly less than 1m deep), on clay and mud. Water is very turbid, with near zero transparency and is devoid of macrophytes, either emergent or submerged. At the time of collection, conchostracans abounded. Five males and seven females collected by K. Martens & L. Hoenson on 7 September. 2001.

Accompanying ostracod fauna: *Isocypris priomena*, *Homocypris conoidea*, *Megalocypris* sp. (juveniles only), 2 species of *Sarscypridopsis* and a new genus and species of Cypridinae-Cypridini.

2. Small pan, annex farm dam near Blinkvlei, 8 females and 3 males, collected by K. Martens & L. Hoenson on 7.9.2001 in the Van Rhynsdorp area (coordinates: 31° 43′ 29″ S, 18° 55′ 28″ E).

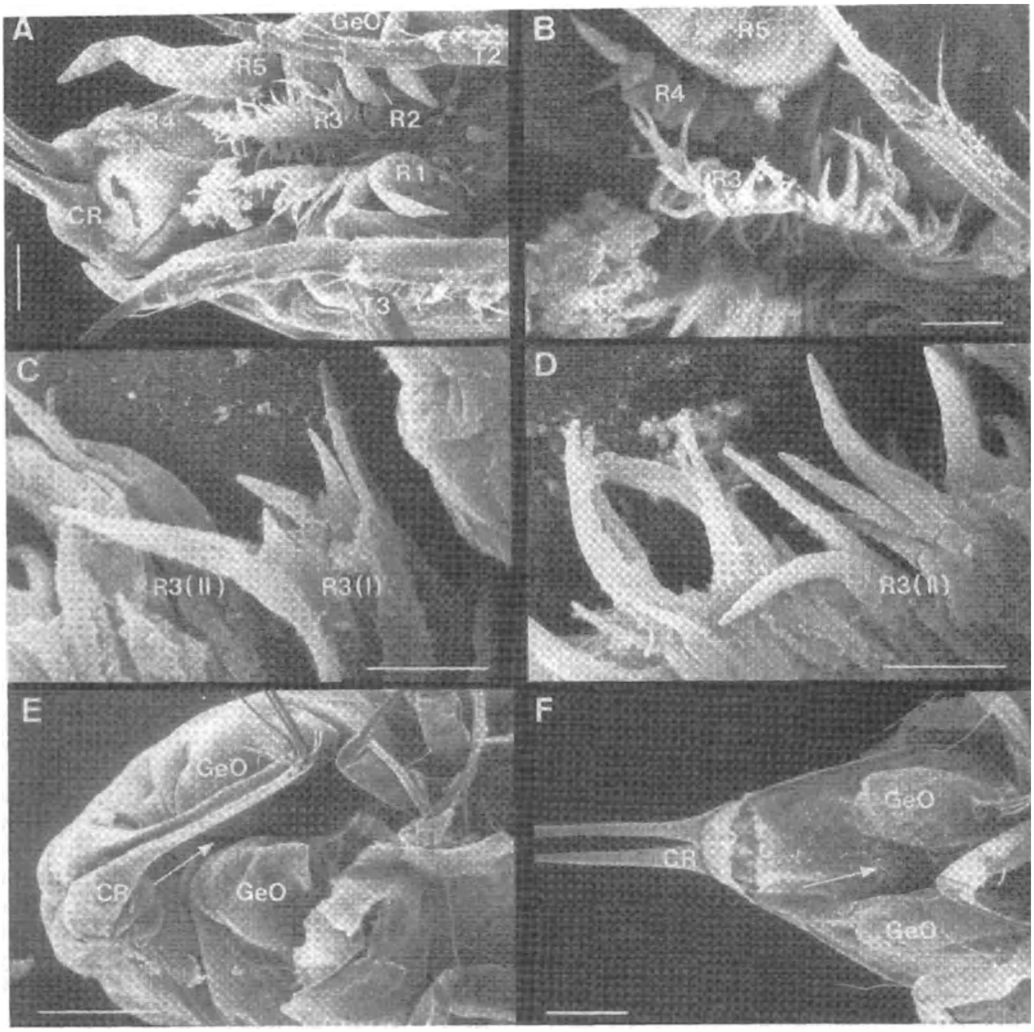

Figure 7. Liocypris grandis Sars A–D, female, F, (A-1) female and *Megalocypris* sp. (E). *L. grandis*: (A) Ventral view of caudal part of adult female body, showing paired appendages R1–R5. (B) Idem, detail of R3 (palp and main body). (C) Idem, detail of palp of R3. (D) Idem, detail of rays of main body of R3. (F) Female (A-1), Ventral view of caudal region. *Megalocypris* spec.: (E) Ventral view of caudal part of adult female, showing absence of additional paired appendages. Scales (in μm): A = 200; B = 100; C = 50; D = 50; E = 500; F = 200.

Accompanying ostracod fauna: *Isocypris perangusta*, *Homocypris conoidea*, *Megalocypris* n. sp., *Sarscypridopsis* sp., and a new genus and species of both Cypridinae-Cypridini and Cypridopsinae.

Several specimens from both localities were used for the present illustrations and descriptions. They are curated in the collections of the Royal Belgian Institute of Natural Sciences (Brussels, Belgium) and the South African Museum (Cape Town, RSA).

Derivation of name:
named after the large size of the animals.

Diagnosis: mostly as for the genus
Valves highly arched, in females with straight, sloping caudal margin (rounded in males), with highest point

in posterior 1/4–1/5; LV with anterior inner list parallel to the anterior margin, not submarginal. In males, ventral valve margin of RV widely diverging from selvage, set with small tubercles. Rome organ on A1 small. A2 with natatory setae very short. Prehensile palps (male) very asymmetrical, distal segment of right palp subquadrate, with concave distal margin and long, pointed ventral tip, left palp with sickle-shaped second segment, slightly swollen in the middle. Hemipenis large, lateral shield with 3 large and prominent lobes, with pronounced three-dimensional structure, medial shield simple, bluntly pointed. Genital appendages in adult female as described for the genus (putative specific differences unknown).

Redescription of male

Valves (Figs 2C,D) elongate, highly arched in the posterior part, dorsal margin strongly sloping towards the front; anterior margin narrowly rounded, ventral margin sinuous, posterior margin widely and asymmetrically rounded, passing into ventral margin without obvious corner. Anterior calcified inner lamella wide, posterior calcified inner lamella developed along ventral side only. Right valve (Figs 2I,J,L) with strongly developed selvage, widely displaced along the ventral margin, submarginal along anterior and posterior edges; posterior part of ventral valve margin crenulated, produced in a blunt angle. Left valve antero-ventrally and postero-ventrally with isolated parts of an inner list (Figs 2K), posterior part of ventral margin furthermore with a sleeve to accommodate fitting structures in right valve. Carapace in dorsal view narrow, greatest width approximately situated in the middle, no anterior overlap, ventrally and posteriorly LV overlapping RV. Muscle scars small (Fig. 2E), and positioned relatively anteriorly, at about 1/3 of the total length, consisting of two fused dorsal scars, two large medial scars (both also consisting of 2 fused scars?) and a small ventral scar; mandibular scars small, rounded, about twice as long as high. External valve surface smooth. Pore canals numerous and branched, about twice as long along the anterior margin than along the posterior margin.

A1 (Fig. 3A) 7-segmented, first segment with one small dorsal and two longer ventral setae. Second segment with small, medio-ventral Rome organ, and very small dorso-apical seta. Third segment unusually elongated (L = c. 5 × width), with one dorso-apical and one ventro-apical seta, both of medium length. Fourth segment about twice as long as wide, ventro-apically with one short and one long natatory seta, dorso-apically with 2 long natatory setae. Fifth segment subquadrate (L = c. 1.5 × width), with chaetotaxy as in the preceding segment. 6th segment with 4 long natatory setae, and one smaller (dorso-apical) setae. Terminal segment small, with two long natatory setae, one long aesthetasc Ya and one seta of intermediate length (c. 4/5 of aesthetasc).

A2 (Figs 3B,B',C) with length of aesthetasc Y c. 1/3 of length of ventral side of first endopodal segment, natatory setae very short (progressively longer from 1 to 5) and with 4 t-setae. Sexual dimorphism of distal chaetotaxy pronounced. On penultimate segment: z3 a long seta, z2 and z1 long, slender claws; G2 a long, stout claw, G1 and G3 short claws. On terminal segment: Gm short, less than half of GM, the latter set with a row of long, stout claws; aesthetasc y3 short, about the same length as accompanying seta, seta 'g' smaller still.

Md-palp (Fig. 4A,A') on first segment with 2 s-setae relatively short, one stout and one slender, alpha-seta with proximal 2/3 tapering, distal 1/3 flagellum-like, 4th seta smooth, as long as the s-setae; respiratory plate with 5 apical and one lateral respiratory rays of unequal length. Second segment with 3 dorsal setae, two longer, subequal, one shorter; ventral side with a cluster of 5 setae: 3 long and smooth, one shorter and hirsute, beta-seta relatively narrow, hirsute. Third segment dorsally with a group of 4 smooth, subapical setae, one long, two shorter and subequal, a fourth about half the length of the longest one; medially with one stout and hirsute gamma-setae, ventrally of which three slim, but longer setae are situated, ventrally with 2 smooth, subapical setae, one long and one shorter (less than half the length of the longer one). Terminal segment about twice as long as basal width, tapering towards the distal side, apically with 3 long and 4 shorter setae, all smooth.

Mx1 (Fig. 3E) with palp two-segmented, first segment elongate, with 5 dorsal-apical and 2 subapical setae; second segment rectangular, about 1.5 times as long as the basal width, apically with 6 setae, two of which longer and claw-like. Third endite ventrally with one medio-lateral seta, dorsally with one hirsute subapical seta; apically with 8 smooth setae, two of which large and stout *Zahnborsten*, also smooth. Second and third endite without special features. Basipodite of T1 (Figs 4D,E) with 2 short and smooth unequal a-setae, 1 longer, hirsute b-seta and one shorter d-seta, hirsute in its distal half; apically with about 10 apical and 4 subapical setae of varying length and appearance. Exopodite (branchial plate) consisting of

6 hirsute rays. Endopodite a 2-segmented prehensile palp; right palp (Fig. 4E) the largest, first segment distally dilated, ventrally with 2 small, subapical sensory organs, distal segment subquadrate, with concave distal margin and long, pointed ventral tip carrying elongated sensory organ; left palp (Fig. 4F) with first segment with rounded lateral sides, distal margin narrow, ventrally with one subapical protuberance carrying two minute sensory organs; second segment sickle-shaped, slightly swollen in the middle, distally with one robust sensory organ.

T2 (Fig. 4G) a walking limb, with seta d1 about three times the length of d2 and penultimate segment divided, all segments elongate.

T3 (Fig. 5C) a cleaning limb, distally with a distal pincer-organ, consisting of fourth segment fused with distal part of third segment.

Caudal rami (Fig. 5B) slender and symmetrical, with two slender claws and 2 short setae.

Attachment (Fig. 4C) slender and without loops.

Hemipenis (Fig. 5A) large, lateral shield with 3 large and prominent lobes, with pronounced three-dimensional structure, medial shield simple, bluntly pointed. Inner anatomy simple, with elongate labyrinth, post-labyrinthal inner spermiduct simple, without extra coils and surrounded by sclerotised structures.

Zenker organs (not illustrated) large and well-developed, with indeterminate number of spinous whorls.

Redescription of female

Valves (Figs 2A,B) with pronounced sexual dimorphism, elongate, highly arched in the posterior part, dorsal margin strongly sloping towards the front; anterior margin narrowly rounded, ventral margin slightly sinuous, less so than in the male, posterior margin straight and passing into ventral margin with an blunt angle. Anterior calcified inner lamella wide (Fig. 2G), posterior calcified inner lamella developed in postero-ventral corner only (Fig. 2H). Selvage submarginal along anterior, posterior and ventral margins. Left valve antero-ventrally with part of an inner list, posteriorly without inner list. RV without inner list on either posterior or anterior side. Both valves with a three-dimensional structure in posterior third of ventral margin, most likely interlocking. Carapace, muscle scars, external valve surface and pore canals as in the male.

A1, Md, Mx1, T2, T3 and caudal rami as in the male.

A2 (Fig. 3D) largely as in the males, but with chaetotaxy of last two segments as typical of female Cyprididae, i.e. three z-setae (z1 the shortest), claws G1 and GM the longest, g3 only slightly shorter, Gm about 2/3 of length of GM and G2 short, less than half the length of G1. Aesthetasc y3 slightly shorter than accompanying seta, seta ‘g’ slightly longer than claw Gm. T1 (Fig. 4B) with large and stout palp, distally with three relatively short setae, central one the longest, lateral ones less than half the length of the central one; exopodite with 5 rays, basipodite as in the male.

Female reproductive organs large and symmetrical, with a series of additional appendages medially associated with these organs. These are called R-appendages (see discussion). Appendage R1 (Figs 5D, 6A,C,D,F and 7A) consists of a paired, well-sclerotised, plate-like structure, with a rounded dorsal and straight ventral margin, anteriorly bluntly pointed, posteriorly with a long, elongated point. R1 is concavely rounded towards the antero-lateral sides.

R2 (Figs 5E, 6A, C, D, F and 7A) is closely positioned to R1, being inserted posterio-medially of its attachment. It is a worm-like, tubular structure, not well-sclerotised, distally bluntly pointed and with apparent pseudo-segmentation, not continued internally.

R3 (Figs 5F, 6A, D and 7AB–D) resembles a true appendage more closely than any of the other R-appendages, as both a palp-like (I) and a respiratory plate-like structure (II) can be identified. Nevertheless, even in this appendage there are striking differences with other cephalic or thoracic ostracod appendages. For example, structure II resembles a respiratory plate of the Mx1, but whereas the latter has a unique row of hirsute rays around the plate, the rays in structure II of R3 are not arranged linearly, but rather in unclear subgroups, can be bifurcated, and are completely smooth. Palp (I) and plate (II) are also incompletely separated.

R4 (Figs 5G and 6A, D, E) has a rod-like morphology, with pronounced pseudo-segmentation (in some cases, this segmentation is almost completely continued internally) and is distally pointed. R4 is hollow up to the most distal point.

R5 (Figs 5H, 6A and 7A) strongly resembles a (thoracic – see below) limb *Anlage* as illustrated for other Cyprididae (Smith & Martens, 2000). It is inserted close to, but separate from, the caudal corner of the genitalia. The structure is hollow, has about the same size as a full-grown T2 and points in a caudal direction.

None of these additional structures R1–R5 has any internal musculature.

Abdomen and thorax of adult females dorsally and ventrally without remnant traces of segmentation.

Remarks:
Only few specimens were available to Sars (1924) for the original description (see above, type material) and he did not illustrate the sexually dimorph male valves, nor did he describe the R-appendages, apart from the large R5, which he named 'genital lobe', in analogy of such structures in, amongst other groups, the Megalocypridinae.

Liocypris grandis is the only known species in the Cyprididae, and indeed also in the Podocopida, where adult females have such additional appendage-like structures associated with the reproductive organs. These appendages appear after the final moult only; (A-1) juvenile females show no trace of them (Fig. 6F). No remnant traces of segmentation on the body of the adult females (either dorsal or ventral) were found, so that none of the R-appendages can be directly linked to segments.

Measurements
Following Sars (1924): length of adult female up to 4.40 mm.
New measurements (in mm):
Female (KM.3242): LV, L = 5.17, H = 2.63. RV, L = 5.05, H = 2.50.
Female (KM.3113): RV, L = 4.92, H = 2.46.
Male (KM.3112): LV, L = 4.97, H = 2.68. RV, L = 5.22, H = 2.80.
Male (KM.3243): LV, L = 4.83, H = 2.70. RV, L = 4.84, H = 2.76.

Relationships
In all soft part structures, *Liocypris grandis* is a typical member of the Cyprididae; the absence of any relevant known feature (such as flagellated claws in A1 (Megalocyoridinae), Triebel's loops in the attachment of caudal rami (Cypricercinae), a 'c'-seta on the T1, additional coils in the hemipenis (Eucypridinae), etc. would make it difficult to lodge it into any of the extant subfamilies. The presence of the additional R-appendages in adult females, however, puts the genus and species into an isolated position in the family and indeed necessitates the erection of a new subfamily. Superficially, there is some resemblance between *Liocypris* on the one hand and *Amphicypris* on the other. However, the latter genus does not have the additional R-appendages and has other structural features which determine its taxonomic position (the classification of the latter group will be discussed elsewhere).

Ecology and distribution
Liocypris grandis was first described by Sars (1924) on specimens collected from a vlei near Stompneus, a small community on a peninsula c. 100 km north of Cape Town. Since the original description, this large and conspicuous ostracod was never reported again. I went to re-sample the type locality in 1987, but found that it was, in all probability, destroyed by the construction of a small airport near the village of Stompneus. The species did not occur in any of the other surrounding vleis sampled at that time, and I considered that it might even have been extinct. This prompted me to suggest *Liocypris grandis* for inclusion in the Red Data List of endangered South African invertebrate taxa. The present rediscovery of the species in more northern localities shows that *Liocypris grandis* is not a point-endemic and has a wider distribution, at least within the northern part of the Western Cape Province.

Both the original type locality and the new localities described above are predator-poor, temporary habitats in arid regions, which are periodically inundated (most likely for some months in most years). Both new localities had rather muddy substrate and turbid water. The animals apparently were living in or on the mud, not on hard substrates in the water, such as logs and branches. Females appeared to be a bit more abundant than males; at the time of collecting some (A-1) juvenile stages were present together with the adult, fecund individuals.

Discussion

The presence of these 5 additional paired structures is unexpected and several question arise. What are they? Do they belong to the body plan of this ostracod or are they alien structures? If they are part of the ostracod itself, are they then appendages or are they something else?

First, I argue that indeed these structures belong to the ostracod and are not, for example, parasites. The reasons for this are that are perfectly symmetrical, occur in the same morphology and position in all adult females investigated (both in the type specimens from the 1920s and in newly collected specimens from 2001) and are absent in juveniles, adult males and other ostracod species, some of which also giant (see

Megalocypris spec., Fig. 7E). Secondly, these structure are believed to be homologous to appendages, because of their paired aspect, and because of the morphology of R3 and R5 (see below). Morphologies of R1, R2 and R4 are nevertheless highly unusual.

Finally, it is here accepted that the 5 additional, paired appendages in female *Liocypris grandis* are associated with the female genitalia, firstly because of ontogenetic reasons (all appendages appear in the final moult only) and secondly because of their position: they are either inserted medially of the female copulatory organs (first 4 appendages) or directly posterior to the female genital organs (fifth appendage) (Figs 6A,B and 7A). Therefore (and because no direct homology is possible – see below), the individual paired structures are here referred to as R1 – R5 (R for reproductive), from anterior to posterior.

Homology of the R-appendages

Liocypris grandis is the only podocopid ostracod thus far known with such additional appendages in adult females. If the hypothesis that space constraints in small, bivalved arthropods caused the dramatic reduction in number of appendages holds true, than one could postulate that the gigantic size of *Liocypris grandis* (L = 5 mm or longer) is the underlying reason for the occurrence of the additional appendages in this species. They occur simply because they can, as there is enough space. However, close examination of an even larger cypridinid ostracod (L = 7–8 mm), *Megalocypris* sp. from a pool close to the new localities of *L. grandis*, showed the complete absence of R-appendages in this species. The size of *L. grandis* is not the only reason for the occurrence of the R-appendages.

R3 can be homologised with a biramous appendage as a palp (endopodite?, structure I) and a respiratory plate (exopodite?, structure II) occur. It is thus the most similar to a 'normal' ostracod appendage. R5 resembles a giant (heterochronic) limb *Anlage*. The other structures are morphologically totally unrelated to any ostracod, or indeed any known crustacean appendage. Their homology is therefore intriguing.

The additional appendages only occur in the adult female and are absent in the (A-1) female juveniles. As this is typical of reproductive structures, it is here accepted that these additional appendages are fully associated with the female reproductive organs and might give indications regarding the number of (paired) appendages that gave rise to the female copulatory organ. If these structures are indeed paired and biramous appendages, then they could be homologised with anything between five and three pairs of appendages. When all five structures are considered separate appendages (with only R3 showing indications of the biramous aspect), then they represent five segments. If R1 and R2 form one pair (endo- and exopodite), R3 forms a second appendage and R4 and R5 are kept separate, then four segments are involved in the formation of this genitial complex. If, finally, R4 and R5 are also considered exo-and endopodite of one biramous appendage, then only three segments are involved. The latter is not very likely, as the *Anlage* of (originally biramous) thoracic limbs typically consist of one structure like R5 only. Any of the above homology-scenarios are of course entirely speculative and since no ontogenetic series can be followed, will most likely remain so. However, if correct, it would mean that the female reproductive organ in this group consists of a fusion of four to five pairs of appendages, representing four to five segments.

Further on the putative homology of these appendages, it can be seen that they (as well as the female reproductive organ) are situated anterior to a structure which resembles the 'sternum' of Kaufmann (1896). The sternum (here called sternum I) was thought to indicate the border between head and thorax (Martens, 1990), at least in the Limnocytheridae. If the present structure in *Liocypris* (here called sternum II, 'St' in Fig. 6D) is the boundary between thorax and abdomen, then both reproductive organs and associated R-appendages are all thoracic in origin. The shape of R5 fully conforms to the shape of the thoracic limb-*Anlage* (Smith & Martens, 2000: Fig. 21), and this can be seen as further support for the thoracic affinities of the R-appendages. Of course, the morphology of an abdominal limb *Anlage* in Ostracoda is unknown, so the possibility that the R-appendages and the copulatory organs are abdominal cannot be fully excluded.

Swanson (1989, 1990) described the presence of 8 limbs (+ caudal rami) in the punciid ostracod *Manawa* (then with uncertain position, recently lodged in the Palaeocopida as their only living representatives – Horne et al., 2002) and tentatively named the 8th limb 'uropod'. He found that the male copulatory organ was fused at the base with this 8th limb on one side. Female genitalia were thought to be elongated lobes, anterior to the same 8th limb, but in this case were associated with "enigmatic, long, fine setae" (Swanson, 1990: 239). Cohen & Morin (1993) homologised the paired male copulatory limb in Cypridinidae (Myodocopida) with an 8th limb, most likely thoracic, and later (Cohen & Morin, 1997) extended this homology to the female genital limbs. In all of these cases, the copulatory organs were either described as being associated with or were fully homologised with only one pair of somatic appendages. An analyses of trunk segmentation and origin of male and female copulatory appendages in podocopid lineages was performed by Tsukagoshi & Parker (2000). They concluded that podocopid ostracods originally had 11 trunk segments (this body plan still occurs in the Platycopida (Schultz ,1976) and in the punciids – Swanson, 1989, 1990), that the first to the sixth segment (T11–T6 when numbered from the caudal side) are thoracic and that the 7th to the 11th segment (T5–T1) are abdominal. Male copulatory organs are believed to be associated with the 10th trunk segment (T2 = abdominal), while female copulatory organs are said to be derived from the 5th segment (T7 = thoracic). As T11 (the first trunk segment) is supposed to be devoid of limbs, thoracopods T1–T3 (limbs 5, 6 and 7) are associated with the 2nd to the 4th trunk segment (T10–T8) and in that case the female copulatory appendage on the fifth trunk segment (T7) is indeed derived from the 8th limb, as postulated by Cohen & Morin (1997). The more caudal homology of the male copulatory organs, as observed by Tsukagoshi & Parker (loc.cit.), agrees well with the postulated inclusion of caudal rami in male copulatory appendages in a number of Cytheroidea, for example in *Limnocythere* (see Martens, 2000) and *Gomphocythere*, (see Park & Martens, 2001; Martens, in press) and in Bythocytheridae (Horne et al., 2002). Martens (in press) analysed the female abdominal structure in a number of *Gomphocythere* species from Lake Malawi (Africa), and found that there is a paired structure consisting of a 3 hirsute lobes and 2 setae associated to the female copulatory organ. This was thought to be homologous to the caudal rami, but could very well constitute one or more paired appendages. The thoracic homology of the female copulatory organ, i.e. relatively anterior on the trunk, would indeed allow for the presence of other (abdominal) appendages.

Homologies of copulatory appendages in Cypridocopina are completely lacking. The present discovery of the additional paired R-appendages, associated with the female copulatory appendage, throws doubt on the homology of these structure with a single pair of somatic appendages in other Ostracoda. Although at present the female copulatory organ is indeed one structure, associated (also ontogenetically) with the 5th trunk segment, its homology to more than one pair of appendages and segment can apparently not be excluded

Origin and function of the R-appendages and phylogenetic position of Liocypris

The phylogenetic significance of these appendages is equally enigmatic: are they relicts of an ancient situation in which stem-group Podocopida had more (thoracic) appendages or in which the appendages forming the reproductive organ were still individually recognisable? Or are these structures apomorphic, newly-arisen and functional morphologies? This question can only be fully discussed when a trustworthy phylogeny exists. A molecular phylogeny of the Cyprididae is presently being built and will be presented elsewhere (Schön & Martens, in prep.). Meanwhile, however, some relevant circumstantial data can briefly be discussed. If the R-appendages were to reflect a plesiomorphic condition, then *Liocypris* should most likely have an ancestral position in the phylogeny of the Podocopida in general. It thus follows that the Cyprididae, *ergo* the Cypridocopina, should be one of the earliest groups within the Podocopida. Following the descriptive, not analytical, phylogram of Martens et al. (1998), based on Maddocks' (1982) data, this is not the case as at least Cytherocopina and Bairdiocopina are presumed to be older. For neither of these groups has anything remotely resembling the R-appendages been reported. Also, Palaeozoic Cypridocopina are quite different from Mesozoic ones, for example in number and shape of muscle scars (Moore, 1961), the Cretaceous *Pattersoncypris* (c 100 million years) being the first ostracod known with the typical appendage morphology of the Cyprididae (Bate, 1972; Smith, 2000). Since *Liocypris* is a giant temporary pool animal, its fossil record is scarcer than for lacustrine ostracods, because temporary habitats have, by their very nature, a much poorer fossil record. The only fossil report of a congeneric species, *Liocypris crassa* Grekoff, 1957, dates from the Upper Jurassic/Lower Cretaceous (140–150 million years) of the Congo Bassin. *Liocypris crassa* could belong to the same genus, but this is far from established, as its shape is rather different and the valves are less than 1.4 mm long, compared to 5 mm or more in the extant *L. grandis*. If *L. crassa* and *L. grandis* are indeed congeneric, then this goes a long way in corroborating the present scenario describing *Liocypris* as an old group, closely related to the ancestors of the present-day Cyprididae. A second possibility, in which the R-appendages, but not *Liocypris* itself, show ancestral affinities, is that the R-appendages may have been phenotypically lost, but genetically preserved in the lineages leading up to *Liocypris*, and that they were expressed again in the latter group. Similar scenarios on such dormant genes have been described for other arthropods. Fryer (1999) suggested such an evolutionary pathway for the extant (atavistic) individuals of the one-eyed brine shrimp, which would reflect the (ancestral) morphology of a possible Cambrian species. Recently, Whiting et al. (2003) described a series of 'wing loss' and 'wing gain' events in the history of stick insects. Although time frames for these events are not absolute, the latter case is well documented. If the R-appendages represent a case of switched-on dormant genes, the phylogenetic position of *Liocypris* within the Cyprididae is largely irrelevant.

Thirdly, the R-appendages could constitute a recent and functional adaptation. Also in this case, *Liocypris* can have almost any position in the phylogeny of the Cyprididae, from relatively ancestral to highly derived. One can thus only test this hypothesis on its internal consistency. If the R-appendages are newly-formed structures, than they are most likely adaptations. Since these structures are associated to the female reproductive organs, their function should be related to reproduction. There are two obvious possibilities. Firstly, these appendages could be used to handle eggs during brooding, for example to expose eggs and embryos to optimal oxygen levels. Given what little is known about the autecology of this animal, which is found in presumed low oxygen conditions of shallow temporary subtropical ponds, this is a distinct possibility. However, brooding is not established in this species (nor in any other Cyprididae for that matter – Horne et al., 1998) and the need for 5 appendages with such elaborate differentiation in structure for this behaviour remains unclear. Also, the absence of any musculature in R1–R5 seems to exclude their use in handling eggs and embryos. Alternatively, the appendages could be involved in mate recognition and form part of morphological module of the Specific Mate Recognition System (SMRS) in this species. There are several problems with this. Firstly, this would invoke male mate choice, as the female has the specific morphologies here, and this would be the first documented case in podocopid ostracods. Secondly, one would only expect this in a speciose genus and although several species of *Liocypris* might have existed sympatrically in the past, the genus is at present monospecific. It is important to point out that even if the R-appendages are functional, they could still be homologous to the ancestral structures which formed the female copulatory organs.

The phylogenetic position of *Liocypris* in the cladistic molecular analysis of the Cyprididae will only partly provide a test for these scenarios. Behavioural analyses of this species, during copulation and egg-handling, will also be required to discriminate between the above possibilities.

Conclusions

The enigmatic giant ostracod species *Liocypris grandis* has five additional pairs of appendage-like structures associated with the (adult) female copulatory organs. These structures most likely represent four to five ancestral appendages and segments. This suggests that the female copulatory organs in podocopid (or at least in cypridocopinid) ostracods might be homologous to more than one pair of thoracic appendages, as was thus far believed. The R-appendages could either be atavistic relicts of such ancestral structures, or their morphology could be adaptive. Since they appear only during the final moult, their function would then most likely be related to reproduction, for example for handling eggs and embryos during brooding or in mate recognition. For various reasons, neither of the latter two possibilities is very likely. The aberrant morphology of *Liocypris* provoked the erection of a new subfamily within the Cyprididae.

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Morphological analysis of some cryptic species in the *Acanthocyclops vernalis* species complex from North America

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Abstract

Patterns of morphological variation and reproductive isolation were examined for several North American populations of copepods in the *Acanthocyclops vernalis* Fischer A., 1853 (Copepoda, Cyclopinae) species complex. The copepods were collected from six sites in Wisconsin, U.S.A. Morphological analysis of 120 adult females revealed that a character used previously to distinguish species in this group was unreliable because of phenotypic plasticity. Most of the morphological variance was due to environment (Laboratory vs. field) and to field site. Relatively little of the variation was due to measurement error or asymmetry. Multivariate ordination analysis produced poorly-defined clusters of individuals, suggesting that different biological species are difficult or impossible to distinguish using a set of easily-measurable morphological characters. In our study, morphological similarity was independent of geographic distance among sites, between 0.05 and 300 km. Isofemale lines within sites showed little or no reproductive isolation, but nearly complete isolation among sites. Reproductive isolation was also independent of morphology. These results suggest that the *Acanthocyclops* population at each site could be considered a distinct cryptic biological species. These copepods expressed morphological stasis – persistence of morphological uniformity despite reproductive isolation. Because of the effect of site and environment on morphology, we recommend using much larger collections (many sites), common garden experiments, and a multi-disciplinary approach (morphological, reproductive, chromosomal, and molecular) as the basis for future taxonomic research on putative copepod species.

Introduction

Cryptic speciation is a common phenomenon in copepods that has been well studied in marine systems. Examples of groups of morphologically similar species include Frost (1989) and McLaren et al. (1989, seven biochemically and genetically distinct species of *Pseudocalanus* in northern oceans), McKinnon et al. (1992, phenotypically plastic but biochemically different sibling species of the inshore copepod *Acartia* in two adjacent Australian bays), Ganz & Burton (1995) and Edmands (1999, outbreeding depression in populations of the intertidal copepod *Tigrious californicus*), Knowlton (2000 excessive lumping of morphologically similar but genetically distinct marine copepods), and Lee (2000) and Lee & Frost (2002, genetic differences between proximate populations of the coastal copepod *Eurytemora affinis*).

North American freshwater copepods in the genus *Acanthocyclops* with 17-segmented antennae have been interpreted as one or more morphological species (Price, 1958; Smith, 1981; Dodson, 1994). Different geographic populations are remarkably similar morphologically, yet show subtle morphological differences that continue to obsess taxonomists. This pattern of difficulty in describing cryptic species is characteristic of freshwater cyclopoid genera. Enigmatic species swarms have been described for all well-studied and species-rich genera, including, for example, *Acanthocyclops* (Smith, 1981), *Tropocyclops* (Reid, 1991), *Diacyclops* (Reid, 1992), and especially *Cyclops* (Einsle, 1993). Copepodologists are just beginning to use sophisticated genetic and statistical techniques to explore the taxonomic meaning and fitness implications of subtle morphological variation within species complexes (Hoþyñska, 2000; Lajus & Alekseev, 2000).

Dodson (1994) re-described two very similar *Acanthocyclops* species that were distinguished by two characters. *A. vernalis* was distinguished from *A. robustus* by the presence of a patch of spines (P4ANT) on the anterior face of the P4 coxa (see Fig. 1D in this paper and Fig. 4G in Dodson, 1994). A less consistent character was the terminal segment of the P4 endopod, which has two terminal spines (see Fig. 1C in this paper). In *A. vernalis*, the outer spine was described as being often longer than the inner, and the opposite was characteristic of *A. robustus*. Otherwise, no morphological differences were detected between the two species.

Previous work, reported in Dodson (1994), showed that the number of spine-like setae on the P4 endopod terminal segment depended on some environmental factor, probably temperature. Cold water forms were characterized by 5 spines, while warm water forms had only the two terminal spines. This phenotypic plasticity was clearly not useful in distinguishing species, and such variation in one character provided a warning that other morphological characters could be influenced by environmental factors.

As part of a collaborative project, the Dodson (1994) key was used to identify *Acanthocyclops* species used in a series of mating trials between isofemale lines established from adult females collected from 5 of the six sites (Grishanin, unpublished data). As part of the establishment of isofemale lines, it was necessary to identify field-caught specimens as well as individuals in laboratory cultures. In the course of this study, it became clear that the morphological characters being used to separate species were probably unreliable. Specifically, animals from the same site, and even siblings from the same isofemale line expressed presence or absence of P4ANT. While it is possible that two species were present in a single pond, it is unlikely that two species would have the same mother.

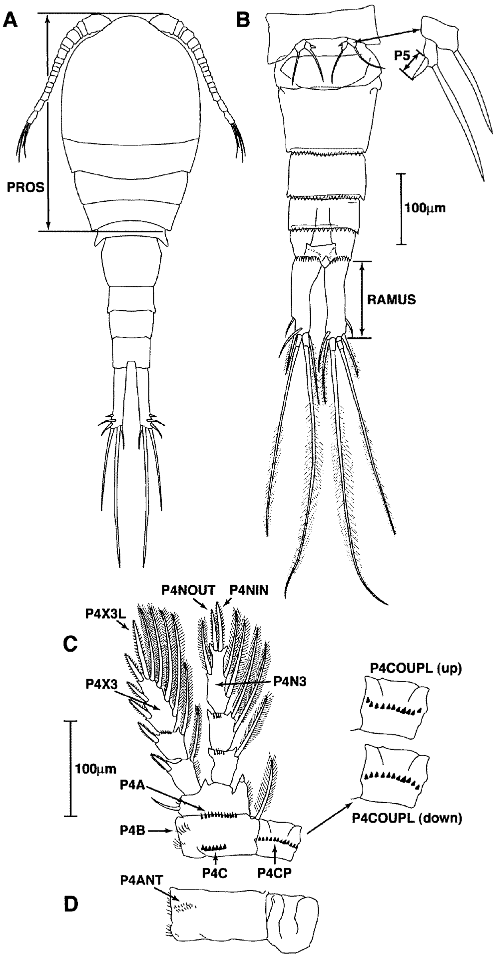

Figure 1. Drawings of the 14 morphological characters used in this analysis. The acronyms are defined in the text. The drawing is representative of a generic member of the *A. vernalis* species complex.

The unreliability of the main distinguishing character produced a desire to know whether it was possible to use morphological characters to describe groups of individuals. Questions that arose included:

- How variable are standard morphological characters used to describe *Acanthocyclops* species?
- To what degree are standard morphological characters correlated? Especially, is there an allometric body-size effect on characters?

- All of the characters show slight variation within a population. How much of this variation is due to effects of environment (field vs. laboratory conditions), site (lake), measurement error, asymmetry, and individual morphological variation?
- Which of the standard characters explain most of the morphological variation?
- Are morphological and geographic distances correlated?
- Is morphological difference correlated with reproductive isolation?

To answer these questions, we measured 120 adult females from the field and laboratory. We were able to take advantage of 48 isofemale lines raised in a common laboratory environment, to address the questions above. Some of these animals were included in mating trials designed to measure reproductive isolation (Grishanin, unpublished data).

Materials and methods

Sites

Copepods were collected in May 2001 from 6 sites in Wisconsin. Three of the sites are in northwest Wisconsin. These are small shallow lakes in Chippewa Co, Lat. 45.2341, Long. 91.1184: 4 miles west and 4 miles north of Holcombe, Wisconsin. Parejko Pond is about 150 m south-southwest of Shorts Pond #1 and Shorts Pond #2, which are only about 50 m apart. Short's #1 is southeast of Short's #2. The remaining three sites are in Dane Co., in south central Wisconsin. Cleveland Road Ditch is a narrow intermittent ditch less than 0.5 m deep when full, at 43.0931° N and 89.6022° W along State Highway 14, near the intersection with Cleveland Road. Trek Pond is a shallow urban retention lake at the Mineral Point exit of Madison's Beltline (Highway 12 & 14) at 43.0606° N, 89.5237° W. Lake Waubesa is a moderate-sized lake in southern Dane Co., at 43.0096° N, 89.6022° W. Copepods were collected in shallow water at the lake's outlet. (Specimens from Lake Waubesa were used in the morphological analysis, but not in the mating trials.) The animals that had developed in the field would have experienced temperatures during their development in the range of 5–10 °C.

Specimens were categorized as:

- Founders – Adult females carrying eggs. These animals were caught in the field, and killed after their young began developing. These animals had been fertilized by one or more males before capture.
- Isofemale Lines: The clutches of Founders were kept isolated and used to start isofemale lines for mating experiments. Male and female offspring were grown from early embryo stage in a common laboratory situation. Lines were maintained by matings among siblings.
- Hybrids – Animals from a few lines that resulted from successful crosses between different lines.

Initially, 48 isofemale lines were maintained at a temperature of about 22 °C, at ambient photoperiod. The animals were fed luxurious amounts of flagellated yellow-brown alga *Cryptomonas ozolini* Sküja from Starr Collection at the University of Texas at Austin (culture number UTEX-LB2194) and newly hatched *Artemia salina* brine shrimp nauplii. Mating occurred readily in the lab and fecundity was high.

After sufficient progeny were produced in the isofemale lines for the purpose of mating trials (see Grishanin, unpublished data), the number of isofemale lines was culled to nine. The present morphological analysis focuses on the founders and their offspring of the nine isofemale lines, along with representatives of 38 isofemale lines not used in mating trials, and 6 specimens from Lake Waubesa.

Adult female specimens were preserved in 70% ETOH. Dissections were done in Hoyer's mounting medium (Dodson and Frey 2001) on microscope slides. The urosome and 4th thoracic segment were separated from the prosome, and positioned so the urosome ventral surface faced up, and the 4th thoracic legs (P4) were flat, undistorted, and with the posterior surface facing up. Dissected specimens were discarded if one or more characters were not visible. A total of 120 individual females were used for data collection.

Morphological character

Morphological characters used in this study are based on those reported in Dodson (1994), with a few additions. Additional characters were added after a careful survey of the appendages, searching for characters that showed promising variation among sites. We favored counts and measurements and avoided shape characters. Morphological characters were measured only on adult females, and were found on the urosome and fifth and fourth thoracic legs. Note that the spine pattern on the basal segment of the fourth thoracic leg is probably related to pre-fertilization mate recognition, and therefore is of potential taxanomic value (Hołyñska, 2000).

Integumental (sub-cuticular) pore patterns on the body have been successfully used to distinguish cryptic species in other copepod general (Bannister, 1993; Baribwegure, 2001). Our specimens were dissected and mounted before we learned of this morphological character. We were able to see the major sub-cuticular pores on the dorsal surface of the urosome, and concluded that variation in this pore pattern was minimal and not useful.

We recorded data for 14 morphological characters (Fig. 1) on each of 120 individuals. The characters included six length measurements and seven counts and one binary variable. Acronyms are given here to assist in interpreting the results tables.

Six Lengths:

- PROS Length of the prosome axis, from the anterior tip to the wing of the segment carrying the third thoracic leg. (The segment for the fourth leg had been dissected off the thorax.) This measurement is the best surrogate for total body length we had available (Fig. 1A)
- RAMUS Length of the urosome, measured along the outside margin (Fig. 1B).
- P5 Length of the subterminal spine of the fifth thoracic leg (Fig. 1B). þ P4NIN Length of the inner P4 endopod terminal seta (Fig. 1C).
- P4NOUT Length of the outer P4 endopod terminal seta (Fig. 1C).
- P4X3L Length of the (inner) terminal spine of the P4 exopod segment 3 (the distal segment) (Fig. 1C).

Seven Counts:

- P4CP Number of spines in the row across the middle of the coupler of the fourth thoracic legs (Fig 1C).
- P4ANT Number of microspinules in the patch of the P4 basipod, on the anterior face (Fig. 1D).
- P4A Number of microspinules in the distal (marginal, 'A') row of the P4 basipod, posterior face. The spinules are in a line, which is often interrupted in the middle with a space (Fig. 1C).
- P4B Number of microspinules in the distal-lateral patch ('B') of the P4 basipod , posterior face. This count did not include setae along the lateral margin of the segment (Fig. 1C).
- P4C Number of microspinules in the proximal row ('C') of the P4 basipod, posterior face (Fig. 1C).
- P4N3 Number of spine-like setae on the P4 endopod, terminal segment (range was two to five spine-like setae) (Fig. 1C).
- P4X3 Number of spine-like setae on the terminal segment of the P4 exdopod. (range was two to four such setae) (Fig. 1C).

One binary variable.

- P4COUPL This character has two states (in our specimens), and was set = 0 if the spines of the P4 coupler were in a simple downward (proximally-pointing) arc; or = 1 if the outer spine on each side was clearly more distal than the adjacent spine producing an upward pointing arc (both states shown in Fig. 1C, as 'up' and 'down').

Observations and measurements were done with a phase contrast light microscope and an eyepiece micrometer. The characters PROS and RAMUS were measured at a magnification of 63× with a resolution of 11 μm. All other measurements and counts were made at a magnification of 400× and a resolution of 1.7 μm.

Variance partitioning

For each character, we partitioned the total variance into variance related to the field-lab dichotomy, and variance related to individual differences, site, symmetry, and measurement. It was beyond the scope of this study to re-measure all animals on both right and left sides, so we collected data for 21 specimens, measuring characters on both sides, and then re-measuring the same specimens again on the right side, several days after the original measurements. Animals were selected to include at least 2 individuals from each site, and both founders and lab animals were included (except for the Waubesa specimens, which were only founders).

Partitioning of variance is a concept that comes from the world of balanced anova designs for only fixed variables. Because of the nature of our data set, our analysis required a mixed model anova (fixed and random effects) with an unbalanced design. For this reason, the analysis reports first the significance of the only fixed effect (ENVIRONMENT: field vs. laboratory specimens), followed by the relative importance of the variances of the random (stochastic) effects. Variance was partitioned by simultaneous maximum likelihood estimation of the variance components using SAS PROC MIXED (2001). This procedure provides the machinery to properly account for a mixture of random and fixed effects (Littell et al., 1996).

Once the effect of the fixed variable has been accounted for, this analysis is relatively insensitive to the number of levels (degrees of freedom) for each of the random independent variables, even though the design is unbalanced.

The SAS output does not lend itself directly to statements like 'Variability within individuals accounted for XX% of the total variability in the data', but it will yield conclusions such as 'For PROS, after accounting for differences among environments, the variability among individuals was comparable to the variability among sites (σ^2 = 43 vs σ^2 = 41, respectively), while measurement variability was much less (σ^2 = 4.6).' Finally, the SAS analysis correctly computes the denominator degrees of freedom for the F-test of environment (it should be the number of individuals, in this case 21 – this is achieved by nesting INDIVIDUAL in ENVIRONMENT).

The model includes five independent variables:

ENVIRONMENT – was designated as a fixed effect, with two levels: field or laboratory.

INDIVIDUAL – a random effect related to individual variation.

SITE – a random effect with six levels, reflecting the six sites sampled in this study.

SIDE – a random effect with two levels (left and right) nested in 'INDIVIDUAL' to measure the importance of asymmetry in measurements. These are measurements on the right and left side of a subset of animals. An index of asymmetry was calculated, using standardized data (see below, multivariate section), on an individual basis, as the difference between the value for the left side and the value for the right side. A perfectly symmetrical animal would have an index value of zero. This index was not used for variance partitioning, but was used as an additional test of symmetry – the average index for each character was compared to zero using a simple t-test.

MEASURE – a random effect with two levels (first measure and second measurement) nested in 'INDIVIDUAL'. These measurements were done on the same structure, but at least two days apart, to make them as independent as possible. RESIDUAL – a random variable used to account for any left-over variance in the model.

The dependent variable in the model is the SCORE for one of 13 measurements in the data set. We used the raw data score for each variable in the analysis of variance. P4COUPL was not included, because it only showed variability according to site. All Trek and Waubesa animals had one P4COUPL state, and the specimens from the other four sites showed the alternate state. The model used to explore variance in this system is:

SCORE = μ + ENVIRONMENT + INDIVIDUAL +
SITE + SIDE (INDIVIDUAL) + MEASURE (INDIVIDUAL)
+ RESIDUAL

The variance estimates quoted in Table 4 are those that provided the best fit to the data, after the fixed effect (ENVIRONMENT) is accounted for. Sometimes the best-fitting parameters occur on the boundary of the allowable parameter space (i.e., the variance is given as 0), even though we can be certain that the true population-level variance must be greater than zero. When a value of zero variance is reported, this is not a sign that the model-fitting has failed, but is a consequence of the empirical simultaneous best estimate of all the variances. The variables PROS, P4CP, and P4COUPL, which have only one value per individual animal, will of course have zero variance for SIDE.

Multivariate analysis

The multivariate ordination analysis used all 120 specimens, but only 11 of the 14 possible morphological characters – three problematic characters (PROS, P4N3, and P4ANT) were removed at this stage in the analysis. The character PROS was removed because all the other counts and measurements were scaled to a standard body size. The characters P4N3 and P4ANT were removed from the data set, because they are known to be phenotypically plastic. The binary character P4COUPL was included in the ordination analysis. Thus, the morphological space was 11 dimensional (14 total characters minus PROS, P4N3, and P4ANT).

Each of the 11 morphological characters was standardized. Within a character, observations were standardized by subtracting the mean and dividing by the standard deviation for that character. Thus, each standardized character had a mean of zero and a standard deviation of 1.0. In other words, after standardization, the characters were equally weighted for the multivariate analysis. The effect of body size was then removed by regressing each standardized character with PROS. The residuals of this regression were taken as the values to be used in the multivariate analysis.

For multivariate analysis of morphological pattern using the 11 characters, we chose the Non-metric Multidimensional Scaling (NMS) technique (described in the PC-ORD manual, McCune & Mefford, 1999). The NMS technique produced a two-dimensional map, which when compared to the other PC-ORD techniques accounted for the largest amount of the total variation among all 120 individuals. We used a Euclidean distance measure, and tried several seed values to minimize the stress value. (Lower stress value indicates better 'goodness of fit'.)

Morphological and geographic distance

The average morphological position in NMS ordination space of nine isofemale lines (those used in breeding trials) was calculated by averaging the *x*- and *y*-coordinates for 3–5 individuals from each isofemale line. Mophological position in multivariate space was based on the standardized measurements corrected for body length. Pair-wise distances among the nine isofemale lines were calculated using the Euclidean equation and software from PC-ORD (McCune & Mefford, 1999).

Geographic distances were calculated using the spherical Euclidean modified for a spherical surface (the URL is http://jan.ucc.nau.edu/ cvm/latlongdist.php). Isofemale lines from the same site were scored as having zero geographic separation. Morphological and geographic distances were compared using Mantel's test (Mantel, 1967) facilitated by Version 3.0 of the R Package (Legendre & Vaudor, 1991).

Reproductive isolation

Breeding trials were done among 9 isofemale lines. Details of the methods of these crosses are given in Grishanin (unpublished data). An average Index of Reproductive Isolation (IRI) was calculated for each cross and its reciprocal. The IRI ranges from zero (maximum reproduction and survival) to 5 (embryos do not develop).

Results

Three additional taxonomic characters showed unexpected variability (Table 1). The character P4ANT, which was expected to be either present or absent within a species (Dodson, 1994), was observed to be variable among siblings. A few isofemale lines included both animals with and animals without spines. Similarly, the character P4X3 appears to be variable at the individual level, with siblings of the same isofemale line having either 3 or 4 spines, including one instance in which a founding female with 3 spines produced offspring with both 3 and four spines.

A third character, PROS, also varied according to ENVIRONMENT, with founding females significantly larger than their laboratory-raised offspring (Table 1). There are only five lines for which we measured both the field-caught progenitor female and two or more laboratory-raised offspring. In all five lines, the progenitor females averaged larger than their offspring, and only 2 of the 16 offspring were larger than their mother. The weighted average shows that offspring tend to be 0.17 mm shorter (prosomal length) than their mothers, or about 17% shorter.

Each of the 14 variables showed some degree of variation in the total data set (Table 2). In general, the coefficients of variation are in the range of 20–30%. The most variable characters were the smallest and hardest to see (P4ANT and P5) and thus the most subject to measurement error.

All the length measurements (RAMUS, P4NOUT, PRNIN, P4X3L, P5) were significantly correlated with PROS, the surrogate for body length (Table 3, the correlation coefficient for $n = 120$, and $\alpha = 0.01$ is about 0.23, Rohlf and Sokal 1981). All the length measurements (with the exception of P5) had an r^2 value of greater than 0.25. The count-variables were less strongly correlated with PROS.

Several of the length measurements (raw data) were also strongly correlated with each other. After the dependent variables had been corrected for the correlation with body length (variables were expressed as the residual of the regression on PROS), length characters were no longer significantly correlated with each other, with the one exception of P4NOUT AND P4NIN.

The total variance for each character (except for PROS) was partitioned among several independent variables (Table 4). ENVIRONMENT (field vs. laboratory) had a significant effect on all the length measurements, and no effect on the counts. After this fixed variable is taken into account, a substantial portion of the remaining variance was due to the factors SITE and to INDIVIDUAL variation. Measurement error and differences between right and left (symmetry) each typically accounted for less than 30% of the variation in the random variables. Exceptions are the trait P4CP, which had over 70% of its variance accounted for by MEASURE, and P4A, which had 56% of

Table 1. Three examples of variable characters: data are given for the first two characters, measured on animals collected in the field (Founders) or from isofemale lines cultured in the laboratory. Prosomal lengths are given for those lines with both the field-collected progenitor and two or more laboratory-raised offspring (n = number of lab animals)

Character	Isofemale line	Founder (field)	Laboratory reared
P4ANT (number of spinules)	021	unknown	0, 9, 10, 11, 11,16, 18
	110	unknown	0, 18
	138	unknown	0, 12
	148	unknown	0, 22
P4X3 (number of setae)	020	3	3,4
	026	3	3,3,4
	361	4	4
	369	4	4
	Trek1	4	4
PROS (mm in length)	020	0.96 mm	0.73 mm ($n = 2$)
	026	1.04	0.80 mm ($n = 5$)
	361	1.07	0.86 mm ($n = 3$)
	369	1.07	0.90 mm ($n = 3$)
	Trek1	0.85	0.84 mm ($n = 3$)

Table 2. The average and variance for 13 measurements from 120 animals (the binary character P4COUPL is not included here). For the measured characters, the units are micrometer units. The variances are partitioned in Table 4. CV = Coefficient of variation, the standard deviation divided by the mean, expressed as a percentage. For other symbols, see Text

	PROS	RAMUS	P4N3	P4CP	P4ANT	P4A	P4B	P4C	P4NIN	P4NOUT	P4X3	P4X3	LP5
Average	71.71	77.57	2.18	12.77	11.94	11.60	8.01	8.43	25.11	25.75	3.33	34.68	6.41
Variance	11.59	17.63	0.67	2.3	27.36	2.26	1.68	2.45	7.12	7.48	0.47	8.96	2.77
CV as%	16	23	31	18	62	20	21	29	29	28	14	28	43

its variance accounted for by SIDE. Trait P4A was the character that showed the least variation among sites (and is therefore probably the least likely to be a valuable taxonomic character).

In some cases, a variance component is listed as zero (Table 4), even though we can be certain that the population-level variance must be greater than zero. A zero value indicates either a small value (less than 1%), or it may just be the best-fitting variance decomposition for these particular data.

The specimens naturally showed some variation between the right and left sides (Table 5), but the average index of asymmetry (difference between sides) was always less than one standard deviation from zero. Thus, individual animals can be asymmetrical, but the population as a whole was symmetrical for each of the quantitative characters.

The two-dimensional NMS ordination graph (Fig. 2), using the 11 morphological characters, accounts for about 85% of the total variation among individuals (in two dimensions). The two NMS axes represent two independent linear combinations of the 11 morphological variables; each axis positions each individual according to covariance and association of the variables (McCune & Grace, 2002).

We have drawn boundaries around clusters of individual from the six sites (Fig. 2). Four populations are virtually congruent and therefore morphologically indistinguishable: Parejko, Shorts 1, Shorts 2, and Cleveland Ditch. The remaining two sites (Trek

Table 3. Pair-wise linear correlation coefficients among 14 morphological variables, including PROSOME, a surrogate body length. Correlation coefficients greater the 0.5 are in bold type

		PROS	RAMUS	P4NOUT	P4NIN	P4X3L	P5	P4ANT	P4CP	P4A	P4B	P4C	P4N3	P4X3
Lengths	RAMUS	**0.90**												
	P4NOUT	**0.71**	**0.71**											
	P4NIN	**0.64**	**0.64**	**0.93**										
	P4X3L	0.49	0.47	**0.62**	**0.65**									
	P5	0.37	0.45	0.15	–0.02	0.06								
Counts	P4ANT	0.21	0.20	–0.07	–0.23	–0.08	0.45							
	P4CP	–0.10	–0.18	–0.05	–0.04	–0.04	–0.21	–0.27						
	P4A	0.09	0.01	-0.02	0.00	0.01	–0.03	0.02	0.18					
	P4B	0.24	0.25	0.07	0.08	0.03	0.16	–0.09	0.28	0.31				
	P4C	0.42	0.44	0.10	0.06	0.02	0.28	0.34	–0.12	0.13	0.26			
	P4N3	0.25	0.22	**0.60**	**0.71**	0.47	–0.23	–0.33	0.05	0.04	–0.01	–0.21		
	P4X3	0.29	0.40	0.27	0.34	0.23	–0.03	–0.19	0.10	–0.06	0.22	0.07	0.30	
Binary	P4COUPL	–0.18	–0.24	–0.39	**–0.61**	–0.37	0.38	**0.52**	–0.16	–0.13	–0.17	0.16	**-0.61**	–0.39

Table 4. Partitioning of Variance for each of 13 characters, each character was measured on the same 21 animals. The binary character P4COUPL is not included in this analysis. Numbers are the percent of the variance due to the random variables, after variance due to ENVIRONMENT (field vs. laboratory-reared) has been accounted for. '0' = less than 0.001; '—' = variance for SIDE not available because the character does not occur on right and left sides

	Lengths						**Counts**						
Characters	PROS	RAMUS	P4NIN	P4NOUT	P4X3L	P5	P4N3	P4CP	P4ANT	P4A	P4B	P4C	P4X3
Fixed variable													
ENVIRONMENT *p*	<0.0001	<0.0001	<0.0001	<0.0001	<.0001	0.0032	0.952	0.251	0.911	.5917	0.0696	0.0750	0.081
Random variables													
Individual	50	10	33	96	98	8	17	28	27	31	16	57	70
Site	47	86	65	0	0	85	75	0	41	10	59	21	10
Measure (Indiv)	3	2	0	0	0	1	0	72	0	2	19	1	20
Side (Indiv)	–	0	2	4	2	5	0	–	29	56	6	20	0
Residual	0	3	1	0	0	1	8	0	3	1	0	1	0

Table 5. Symmetry index (= Left – Right, using standardized data) for each of 11 characters, based on measurements of 21 animals. None of the averages is significantly different from zero. The total data set is 14 morphological characters, but the characters PROS, P4CP and P4COUPL for which there is only one value per individual (no symmetry) could not be included here

	RAMUS	P4N3	P4ANT	P4A	P4B	P4C	P4NIN	P4NOUT	P4X3	P4X3L	P5
Average Index Value	–1.52	0	0.10	0.19	–0.10	0.10	0.95	–0.19	0	0.05	0.14
Standard Deviation	0.73	0	1.84	0.44	0.24	0.24	0.31	0.29	0	0.33	0.22

and Waubesa) are distinct from the first 4 sites, but probably not distinct from one another.

The two strongest correlations between NMS axes and non-plastic characters were for P4COUPL (first axis, $r = 0.80$; second axis $r = -0.54$) and P4NIN (–0.526 and 0.78). These are highly significant correlations ($n = 74$, $p << 0.001$). The pattern of positive and negative correlations with the two axes means

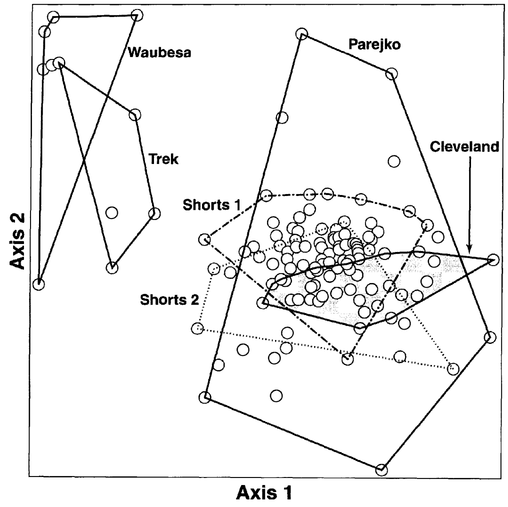

Figure 2. The 2-dimensional graph of the Non-metric Multidimensional Scaling (NMS) ordination. The ordination uses 11 morphological characters, from 120 specimens. Circles represent individuals. Boundaries are drawn around collections of individuals from each of the six sites. Circles in regions of overlapping boundaries indicate individuals that derive from one or the other of the two sites. All individuals were raised under 'common garden' conditions, except for the Lake Waubesa specimens.

Table 6. Linear correlation coefficients (Pearson's 'r') of each of 13 morphological variables with the 1st and 2nd axes of the NMS multivariate analysis. N = 120 individuals. Note that the variables are standardized according to mean, variance, and body length. The critical value for the correlation coefficient (á = 0.1, 119 df, uncorrected for multiple comparisons) is 0.179

Character	Axis 1	Axis 2
RAMUS	–0.001	0.016
P4NIN	–0.526	0.776
P4NOUT	–0.319	0.696
P4X3L	–0.283	0.613
P5	0.620	–0.482
P4CP	–0.503	–0.052
P4N3	–0.574	0.714
P4ANT	0.722	–0.490
P4A	–0.218	–0.321
P4B	–0.350	–0.428
P4C	0.277	–0.484
P4X3	–0.506	0.188
P4CPL	0.796	–0.544

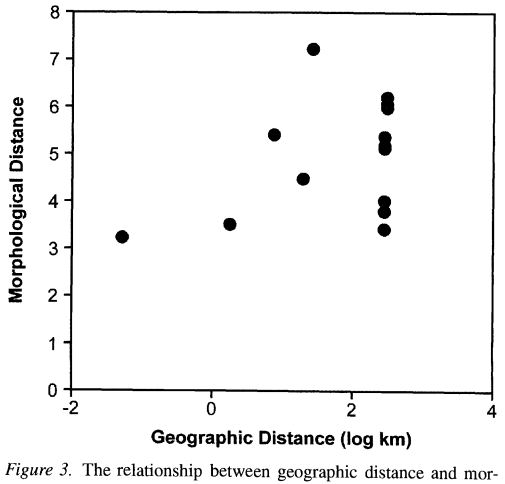

Figure 3. The relationship between geographic distance and morphological distance.

that specimens with the 'up' condition of P4COUPL tended to have short terminal spines (P4NIN) and specimens with the 'down' condition of P4COUPL tended to have long spines (P4NIN). The r^2 values for these two characters are 64–28%, suggesting that there is still a lot of unexplained morphological variance. This is the situation mentioned in the Introduction: great morphological similarity with some tantalizing but slight differences.

Because Figure 2 represents the large majority (85%) of the total morphological variation using just two dimensions, it makes a good visualization of the average morphological distances of individuals from different sites. A comparison of 2 dimensional morphological and geographic distances using Mantel's test and the data in Table 6 showed no significant correlation ($n = 36, p = 0.26$).

The Index of Reproductive Isolation (IRI) values tend to be zero (no reproductive isolation) for different isofemale lines within a site, and for crosses within an isofemale line (Table 6). For crosses between lines from different sites, there was not a significant correlation between IRI and geographic distance (regression analysis, $p = 0.09$, $n = 16$) or for IRI and morphological distance ($p = 0.78$).

Discussion

This study revealed cryptic speciation in the *A. vernalis* species group, with little correlation between morphology and reproductive isolation. Characters traditionally used for systematics in this species group are subject to environmental plasticity. In addition, we found no correlation between morphological and geographic distances.

Body length is not a particularly useful taxonomic character, because it depends on environmental factors such as temperature and nutrition. On the other hand, correlated variables that are independent of body length and environment could be taxonomically useful. Our results failed to produce groups of correlated characters, once the effect of body size was removed.

The desired result of ordination analysis is to identify characters that vary in concert, producing clusters of tight and distinct points in multivariate space. What we see in Figure 2 is only a moderate separation of individuals and a small amount of clustering. The two diffuse clusters can be interpreted as evidence for the existence of two morphological forms. These two clusters appear to be separated according to site.

Our results show that both environment (field vs. laboratory) and site account for substantial portions of the variance in morphological characters. The results emphasize the importance of using specimens reared under common garden conditions for systematic studies.

Also, axonomists are encouraged to look at a large number of sites. Six is clearly too few sites to give a general idea of the pattern of morphological variation within this cyclopoid species complex, if such a real pattern exists. For example, in our ordination, are the gaps between clusters of species a general pattern, or are the gaps only due to the small sample size? An ordination based on ten times as many sites might be much more informative, and might be sufficient to allow a taxonomist to draw conclusions about the number of morphological species.

Our data suggest that it is reasonable to continue identifying individuals in the *A. vernalis* complex, without taking into account either measurement error or symmetry. Both these sources of variance tended to be minor, compared to environment and site. That is, it is reasonable to measure a specimen once, and to use data from either the right or left side.

The results of this morphological analysis, especially of the NMS ordination, suggest that it is premature to decide which characters best separate species in this complex. It is clear that body size (PROS), P4ANT, and P4X3L are not reliable, because they vary within isofemale lines.

Three characters are most likely to be taxonomically valuable. P4COUPL and P4NIN are strong candidates, because of their correlation with the two NMS ordination axes. (P4NIN was used in Dodson [1994] as a character that separated *A. vernalis* and *A. robustus*.) P4COUPL is a new character for the *A. vernalis* group, first observed in this study. It is present in all the individuals from Trek and Waubesa, and absent from all specimens of the other four sites. Thus, P4COUPL may be a valuable character, but our sample size is just too small to evaluate how this character varies over the landscape, and how it varies with other characters.

Our sample size of sites is clearly too small to support a general conclusion about the relationship between geographic separation and morphological similarity. We see that sites separated at the scale of m to km tend to be morphologically similar. However, sites separated by 300 km can be just as similar. In our data set, the morphologically most distinct sites are also the furthest apart, but this may well be a result of small sample size.

With further study, we might find that the Trek and Waubesa sites represent populations of a biological species that could be called *A. robustus*, and the remaining four sites represent sites of biological species in the *A. vernalis* species complex. Individuals from these four sites are similar, with overlapping areas of morphological variation (Fig. 2).

Morphological stasis is the phenomenon of the persistence of extreme morphological similarity over time, despite reproductive isolation (Wake et al., 1983). Our copepods appear to be expressing morphological stasis. Other studies have reported a similar situation. Smith (1981) came to similar conclusions for 20 populations representing the *Acanthocyclops* vernalis species group in southeastern Wisconsin. He identified at least 6 reproductive isolates most of which were not morphologically distinct. Smith (1981) reported ambiguous results for reproductive isolation among 7 populations of *Diacyclops bicuspidatus*, but Monchenko (2000) reported three reproductive isolates from a study of four Kiev and Crimea sites. Price (1958) reported 7 reproductive isolates (biological

Table 7. Average pair-wise distances among 9 isofemale lines. The morphological distances are calculated using laboratory animals only. Geographic distances are straight line distance in km. Index of Reproductive Isolation (IRI, from Grishanin unpublished data) for 20 crosses among some of the nine laboratory isofemale lines

Morphological distances		**S026**	**S102**	**S115**	**S130**	**S142**	**S360**	**S361**	**S369**	**Trkl**
	S026	0	1.61	1.43	1.24	1.42	1.68	1.46	1.05	1.62
	S102	1.61	0	0.53	0.47	0.48	0.77	0.45	0.19	1.61
	S115	1.43	0.53	0	0.21	0.07	1.07	0.75	0.72	1.34
	S130	1.24	0.47	0.21	0	0.23	1.14	0.8	0.66	1.21
	S142	1.42	0.48	0.07	0.23	0	1	0.68	0.66	1.39
	S360	1.68	0.77	1.07	1.14	1	0	0.34	0.67	2.34
	S361	1.46	0.45	0.75	0.8	0.68	0.34	0	0.41	2.01
	S369	1.05	0.19	0.72	0.66	0.66	0.67	0.41	0	1.77
	Trkl	1.62	1.61	1.34	1.39	1.39	2.34	2.01	1.77	0
Geographic distances		**S026**	**S102**	**S115**	**S130**	**S142**	**S360**	**S361**	**S369**	**Trkl**
	S026	0	1.8	1.8	1.8	1.8	274.2	274.2	274.2	280.3
	S102	1.8	0	0	0	0	275.4	275.4	275.4	281.6
	S115	1.8	0	0	0	0	275.4	275.4	275.4	281.6
	S130	1.8	0	0	0	0	275.4	275.4	275.4	281.6
	S142	1.8	0	0	0	0	275.4	275.4	275.4	281.6
	S360	274.2	275.4	275.4	275.4	275.4	0	0	0	7.3
	S361	274.2	275.4	275.4	275.4	275.4	0	0	0	7.3
	S369	274.2	275.4	275.4	275.4	275.4	0	0	0	7.3
	Trkl	280.3	281.6	281.6	281.6	281.6	7.3	7.3	7.3	0
INDEX OF REPRODUCTIVE ISOLATION		**MALES ACROSS**								
		S026	**S102**	**S115**	**S130**	**S142**	**S360**	**S361**	**S369**	**Trkl**
FEMALES VERTICAL	**S026**	0			3.8				4.8	
	S102		0	2.5	3.9			3.6		
	S115		3.9	2.4					3.9	
	S130	4.8	5		0	0		3.9		3.5
	S142				0.8	0.8	3.4			
	S360					4	0			
	S361		4		5			0		
	S369	4.8		3.7				0	0	
	Trkl				4.8					0

species) among 30 populations of *Acanthocyclops vernalis* from the Toronto, Ontario region. He claimed some of the isolates were morphologically distinguishable, but the differences were based on what our study has shown to be morphological plasticity. Lee & Frost (2002) found that different populations of the same morphological species (*Eurytemora affinis*) were morphologically similar, even when they were separated by thousands of km. However, these populations were reproductive isolated. Thus, there appears to be considerable evidence for morphological stasis as a common phenomenon in copepods.

The breeding trials are preliminary in the sense that they are not between enough isofemale lines from a wide range of geographic and morphological separation. Generalization is premature, but our IRI results suggest that there is one biological species per site. This is because the IRI values for crosses between strains from the same site, or for the control crosses within isofemale lines were mostly near zero (no isolation). However, all crosses between lines from different sites had high IRI values, independent of geographic separation or morphological similarity. These preliminary results invite more crosses, over a range of geographic and morphological distances.

Observations of Lajus & Alekseev (2000) suggest that three morphologically distinct populations of *Acanthocyclops signifer* live at different points along the shore of a single lake, albeit a very large lake, Lake Baikal. Our results lead us to predict that the morphological differences shown by *A. signifer* are either the expression of phenotypic plasticity resulting from environmental differences, or that they represent reproductively-isolated cryptic species.

Marine examples also suggest a high level of morphological stasis. Knowlton (2000) concluded that in many marine groups, a morphological approach has lead to excessive lumping of cryptic biological or molecular-criterion species. She found that morphologically similar species were often distinct using molecular markers, suggesting the prevalence of morphological stasis. Ganz & Burton (1995) found two reproductive isolates in five Pacific coast intertidal sites. These five populations were all molecularly distinct and morphologically very similar.

It is clear that extensive field and laboratory studies are needed to evaluate the usefulness of morphological characters to the taxonomy of cyclopoid copepods. To make progress in understanding biodiversity in cyclopoid species complexes, we recommend using large sample sizes of individuals per site, scores of sites, and common garden experiments to separate environmental from genetic effects. Results of common garden experiments can also be used to provide data for quantitative genetics analyses, to measure the heritability of different characters, and thus to be able to avoid using plastic characters. Breeding trials are also necessary to understand the relationship between morphological similarity and reproductive isolation.

Our results suggest the following conclusions concerning the *Acanthocyclops vernalis* species group:

- Morphological characters used previously to distinguish species are unreliable because they show phenotypic plasticity between field and laboratory members of isofemale lines.
- These organisms are symmetrical.
- Measurement error is only a small part of the total morphological variation.
- Morphology is conservative among sites and does not reflect reproductive isolation
- Morphological similarity is independent of geographic among sites, between 0.05 and 300 km.
- Reproductive isolation is minimal between animals from within sites, but nearly complete when two sites are compared.
- Reproductive isolation is independent of morphological difference or geographic separation.
- An extreme but consistent interpretation of our data is that the *Acanthocyclops* populations at each site are distinct biological species, with indistinguishable morphologies.
- Because of the effect of site and environment on morphology, we recommend using much larger collections as a basis for future taxonomic research.

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Dumontia oregonensis n. fam., n. gen., n. sp., a cladoceran representing a new family of 'Water-fleas' (Crustacea, Anomopoda) from U.S.A., with notes on the classification of the Order Anomopoda

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Abstract

Dumontia oregonensis, a cladoceran representing a new family in the Order Anomopoda is described from rain pools in the Agate Desert, Oregon, U.S.A. The proposed family, Dumontiidae, is the newest family within Anomopoda that is not just a reshuffling of already-known species. The general appearance of this novel cladoceran is similar to that of members of the family Macrothricidae. However, a detailed examination of the trunk limbs, particularly of the second pair of limbs, showed that the new species lacks the scraper-setae typically observed in all members of the recently erected suborder Radopoda, to which macrothricids belong. Instead, limb morphology suggests a closer relation of Dumontiidae to the family Daphniidae. Dumontiidae appears to be a 'missing' link between the suborder Radopoda and the 'non-radopodid' anomopods. The families Daphniidae, Ilyocryptidae, Bosminidae, Moinidae and the new Dumontiidae are similar in that they lack of typical radopodid setae on the second pair of trunk limbs. Further studies on the limb morphology of non-radopodid cladocerans are required to solve the phylogenetic relationships among the members of the order Anomopoda.

Introduction

The Crustacean Order Branchiopoda is a morphologically heterogeneous group whose component subgroups share a constellation of primitive crustacean features. It probably originated in pre-Devonian times, and still has many extant highly successful representatives, especially in freshwater habitats.

Much recent attention has been directed to determining the position of Branchiopoda relative to other crustacean orders, either by employing the traditional morphology approach (Olesen, 1996, 1998; Olesen et al., 1997) or molecular techniques (Hanner & Fugate, 1997; Spears & Abele, 2000). However, the inter-relationships among the groups comprising the Branchiopoda are still in need of exploration and clarification.

Fryer (1987a,b) proposed a scheme for branchiopod classification, which involved the elevation of the four lower categories forming the former 'Cladocera' into four distinct orders of Class Branchiopoda, namely Anomopoda, Ctenopoda, Onychopoda and Haplopoda. Fryer's scheme is well-supported by his studies of functional morphology (Fryer, 1968, 1974, 1991), but did not deal with the families comprising the Anomopoda.

Dumont & Silva-Briano (1998) conducted a reevaluation of some genera of the family Macrothricidae within the Order Anomopoda. These anomopods represent a heterogeneous and probably non-natural group (Dumont, 1997). Previous to Dumont & Silva-Briano (1998), the unifying character in the macrothricids was the possession of antennules inserted at the underside of the head, which lacks a rostrum (Smirnov, 1967, 1992). Because that morphology was found to be not truly exclusive of macrothricids, Dumont & Silva-Briano (1998) embarked on a search for a more natural classification, in which they concentrated on the morphology of the trunk limbs, continuing their work begun in Dumont & Silva-Briano (1997).

After detailed studies on limb morphology, Dumont & Silva-Briano (1998) showed that some genera, previously included in the Macrothricidae, were unrelated to true macrothricids and represented cases of convergence in carapace-shape and external appearance. The central thesis of their research is that the primitive chydorid-like macrothricids as well as the 'more highly evolved' Chydoridae and macrothricid-like animals form a natural unit, which they named as the new suborder Radopoda. In the present study, we describe a novel crustacean as a new family, which superficially resembles a macrothricid in gross body morphology, but does not belong to the suborder Radopoda using limb morphology.

Materials and methods

The species described herein, *Dumontia oregonensis* n. fam., n. gen., n. sp., was collected on two separate dates from two ponds in the Agate Desert Preserve, Oregon, U.S.A., a site managed by The Nature Conservancy. Some details of these ponds are included in the discussion.

Dumontia was first collected in January, 1998, from pool # 7 of the Agate Desert Preserve (following the classification by The Nature Conservancy of Oregon) by Wayne Fields, Hydrozoology Co., who kindly made 6 mature females available to the authors. The material was originally identified by Dodson as *Macrothrix* sp.

At the request of the authors, Darren Borgias, Southern Oregon Stewardship Ecologist of The Nature Conservancy of Oregon, collected some additional plankton samples from 16 pools in the same Preserve in March 2001, and the species was found in the pond labeled #5 and re-discovered in the pond labeled # 7 (again, following the Nature Conservancy classification).

The specimens provided by Wayne Fields were preserved in 70% ethanol. The live material obtained from Darren Borgias was reared for several days in diluted (95 μS) artificial pond water at 4.5 °C and fed the green alga *Ankistrodesmus*. Animals for microscopic examination were fixed in 80% ethanol for 1 day, and then mounted as a whole in Hoyer's solution and dissected with minute pins. Figures were drawn using a drawing tube and phase-contrast microscopy. Slides will be deposited in the David Frey Cladocera Collection at the Smithsonian Institution, Washington, D.C.

We compared the limb morphology of *D. oregonensis* to that of several anomopod families using descriptions contained in Alonso (1996) and Dumont & Silva-Briano (1998).

Results and discussion

Dumontiidae, New Family

Dumontia oregonensis, *n. fam., n. gen., n. sp.*

Material examined:

Holotype: One individual collected on March 5th, 2001, from Agate Desert Pond # 7 and partially dissected and mounted on a slide in Hoyers medium. Four additional adult females from the same March, 2001 sample, from Agate Desert #7 were dissected and mounted on two slides, two individuals per slide.

Paratopotypes: The other four individuals on two slides are the paratopotypes.

Paratypes include 6 mature female and over 12 immature female specimens preserved in alcohol. The 6 mature females were collected from pond #7 during March, 2001, and 10 mature females and over 12 immature females that were reared from live material collected from specimens from the March sample from pond #7. Paratypes also include 6 specimens collected from pond #5, March, 2001.

These specimens reside in the collection of the U.S. Smithsonian Institution 2029664, NMNH, Washington D.C.

Type locality: The Nature Conservancy Pond # 7 in Agate Desert, Jackson Co., Oregon, U.S.A. The location is just west of White City Oregon, at 42° 25′ 45″ north latitude and 122° 53′ 50″ west longitude.

Etymology of the family and genus: The family Dumontiidae and its type-genus *Dumontia* are named after Dr. Henri Dumont, University of Gent, Belgium,

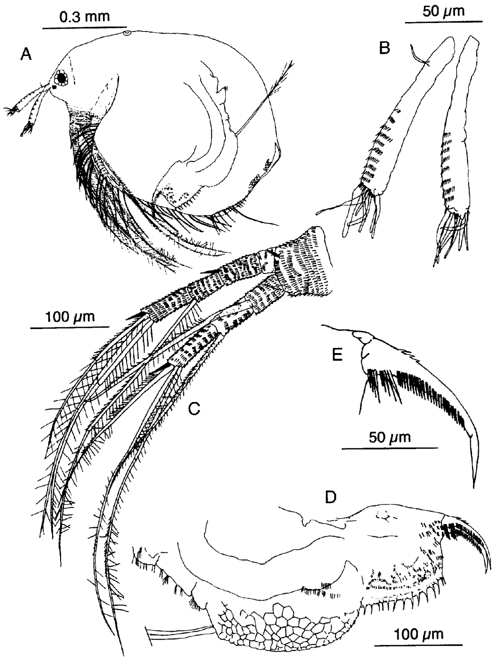

Figure 1. Dumontia oregonensis. (A) Female body in side view; (B) Antennules (A-1); (C) Left second antenna (A-2); (D) Postabdomen; (E) Postabdominal claw.

to honor his extensive contribution to the study of the cladoceran taxonomy and systematics.

Description – parthenogenetic female:

Body: Oval-pyriform to obpyriform in side view (Fig. 1A), pink-colored, 0.85–1.3 mm long. Head markedly reduced. Rostrum extremely reduced, represented by a bluntly conical extension of the anterior margin of head. Eye twice, or more, larger than ocellus.

Valves: Sculptured with conspicuous transverse lines of the kind seen in some members of *Macrothrix*, and small polygons, particularly abundant along and near the ventral margins. Ventral margin of valves flanged with a row of 30–40 long, finely setulated setae, those in the anteroventral corner longer and stouter (Fig. 1A). Similarly long setae are seen in Ilyocryptidae, but in that family these setae are usually bifurcated at several levels. The setae on the ventral row in *Dumontia* diminish in size posteriorly, but the 1–2 posterior-most setae are usually as long as those found in the anteroventral flange (Fig. 4.A), then the row of setae passes into a marginal row of spine-like setae which does not reach to the posteroventral corner. Posteroventral corner of valves relatively low, at level of the mid-longitudinal line of the body, and slightly protruding in side view (Fig. 1A).

Antennules (first antennae): Unsegmented and inserted at the anterior margin of head (Fig. 1B) as in the Macrothricidae, provided with a lateral sensory seta about 1/4 their length from base. The antennules widen towards the distal end, where at least 7 wide, unequally long, aesthetascs arise. The antennules have 8–10 transverse rows of spiniform setules on their distal half, those along the distal margin stouter.

Second antennae (swimming antennae): With setae 1-1-3/0-0-1-3, spines 0-0-1/0-1-0-1; basipodites and segments densely pubescent due to the presence of transverse spiniform setules (Fig. 1C). Natatory setae provided with bristle-like setules; seta arising from the proximal endopodite-segment is stout, unilaterally setulated and slightly curved at the tip. This last seta corresponds to the usually longest natatory seta in *Macrothrix*; but in some *Dumontia* specimens it is relatively shorter than the other setae on the second antennae (see Fig. 1C).

Mandibles: Typical of the Branchiopoda (*sensu* Fryer, 1987) (Fig. 4F, 4G)

Maxillulae: The endopodite has 3 setulated setae on inner face (Fig. 4D); the exopodite is crowned on the distal end with 5 spike-like denticles and a long, setulated seta (Fig. 4E).

P1 or trunk limb 1: (Fig. 2A) This has 2 ejector hooks on the inner side of the corm, the anterior area of corm has a ciliated area between the hooks and the outer distal lobe (ODL) and a long plumed seta. Setae on the four endites are all plumed and long. The three basal endites have 4, 2 and 4 setae, respectively. In front of the main row of setae of E1 and E2, close to the inner margin, there is a plumose seta similar to those found on the second endite (counting from the base). The fourth endite, or inner distal lobe (IDL), has 3 setae, while the ODL has 2 setae, one of them claw-like. There is no discernible gnathobase except for the plumed seta.

P2 or trunk limb 2: (Fig. 2B) The outermost (distal-most) of the series of 6 lobes is regarded as the exopodite, which is adjacent to the oval epipodite (with no setae). The exopodite is provided with an apical seta and a sub-apical, lateral seta. The proximal 5 lobes, or endites, have a total of 15 setae. Counting from the most distal endite, the endites have a = 3, b = 2, c = 3, d = 2 and e = 5 setae. None of these 15 setae is developed into scrapers as found on the

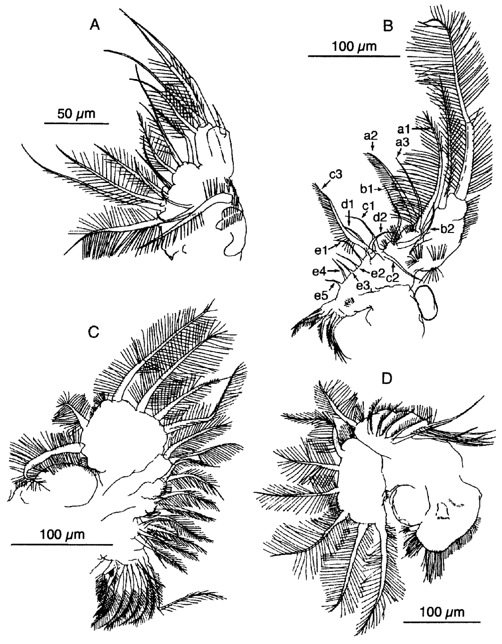

Figure 2. Trunk limbs I–IV of *Dumontia oregonensis.* (A) Limb 1; (B) Limb 2; (C) Limb 3; (D) Limb 4.

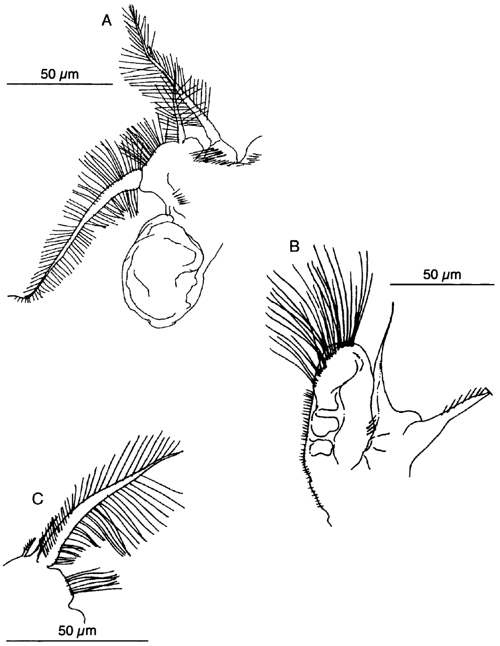

Figure 3. Dumontia oregonensis. (A) Limb 5; (B) Limb 6; (C) Detail of small setae on Limb 2 – the long seta is 3c.

endites of the radopodid cladocerans. Instead, these setae are filiform, with or without setulation. There is a short, bluntly rounded seta between endites 3 and 4 (Fig. 3C). A reduced gnathobase is discerned, with 4 apical setae, different in length and shape, and 6 short plumed setae on the filtering fan. The penultimate of the apical group of setae resembles the scraper-type seta seen in bosminids. (This last type of seta morphology is different from that of the radopodid scrapers).

P3 or trunk limb 3: (Fig. 2C). This has a complex endopodite consisting of 4 fused endites; three of them lobule-shaped and the 4th, closest to gnathobase, much flattened. Each of the endites 1–3 has 2 similarly long plumed setae, accounting for the six setae projecting from the median part of the corm. Some of these setae are accompanied by smaller elements on the inner face of the corm (Fig. 3D). The internal lobe or E4 has a row of 4 marginal setae, plus there is a row of 4 other setae on the inner face of the corm. The exopodite is large and armed with 6 long plumed setae. The gnathobase is armed with 3 setae: one is a crooked and thorn-like seta, and the longest of the 3 is a plumed seta (unaligned with the other 2). In addition, there is a filter comb made up by 9 long setae.

P4 or trunk limb 4: (Fig. 2D). This has a large exopodite armed with 6 plumed setae. A posterior filter comb is represented by 5 plumed setae. The gnathobase is represented by a group of 6 setae on the corm.

P5 or trunk limb 5: (Fig. 3A) This consists of an oval-rounded epipodite with no setae and two lobes with setae. The epipodite is connected to a lobe with two plumed setae near the epipodite. A single plumed seta tops a small inner lobe (which is either the gnathobase or endopod). The seta closest to the epipodite is the longest, the median seta the shortest.

P6 or trunk limb 6: (Fig. 3B) This consists of an oval-elongate lobe, with microspinules along the margin and a tuft of longer setules in latero-apical position. This lobe is adjacent to a smaller lobe from which 2 spine-like setae arise, the proximal-most seta shorter, and non-setulated and abruptly tapered distally.

Postabdomen: This feature is unique in shape; it is long and bilobate, with the distal part almost square (Fig. 1D). The pre-anal dorsal margin of postabdo-

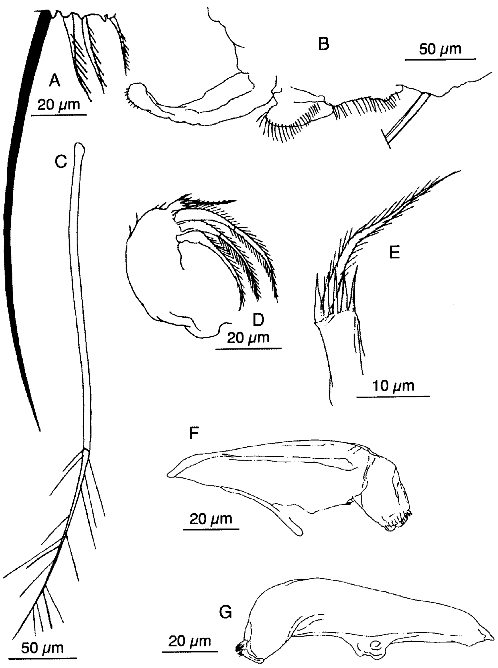

Figure 4. Dumontia oregonensis. (A) Types of setae along the ventral margin of carapace. (B) Posterior part of postabdomen. (C) Natatory setae. (D) Maxillula. (E) Detail of latero-apical area of maxillula. (F) and (G) Mandibles.

men is squamose and smoothly arched, but devoid of marginal spines; the post-anal dorsal margin is almost straight and provided with 13–16, spine-like, inwardly arched teeth. Sides of pre-anal part with sparse rows of setules and reticulated with polygons (mainly pentagons and hexagons). Sides of distal part densely covered with rows of small setules; these setules become larger near the base of claws. The postabdominal claws are long, sometimes sharply curved towards the distal end, with each claw armed with 2 proximal pectens of 4–6 spines diminishing in length proximally (Fig. 1E), and a third row of shorter spines along the median ventral margin (not visible in Fig. 1E). On the ventral margin of the claw, at about 1/4 of their length, there are 2 or 3 setiform to teeth-like projections, resembling the so-called 'Basaldorn' seen in moinids. Natatory setae with proximal part 1.3–1.6 times longer than distal part; setulated distally (Figs. 1D and 4C).

Ephippial female: unknown.

Male: Unknown.

Differential diagnosis: *Dumontia oregonensis* trunk limbs 1 and 2 show a considerable extent of fusion between the endo- and exopodite, to the point where it is often difficult to identify the original building blocks (see Dumont & Silva-Briano, 1998). Trunk limbs 3 and 4 possess simple filter combs. Trunk limb 5 is reduced to an epipodite and a lobe with at least 3 setae all unequal in length. Trunk limb six, where still present as in *Dumontia*, is a vestigial lobe.

The following outline of the limb morphology of the Anomopoda contrasts the non-radopodid taxa, Dumontiidae, and members of the suborder Radopoda. The analysis draws heavily from Dumont & Negrea (2002) and Alonso (1996).

Trunk limb 1.

This limb is 2–3 times longer than wide in daphniids and about as long as wide in radapodids.

(A) Ejector hooks: two at the base of the corm in all anomopods, except Neothricidae. Dumont & Silva-Briano (1998) hypothesized that the ejector hooks are homologous to the basal-most couple of setae of the exopodite in the Ctenopoda, and that the 'ciliated' frontal surface area of the limb between the hooks and the outer distal lobe (ODL) is the incorporated body of the remnant exopodite. Dumontiidae has both ejector hooks and an anterior ciliated area. These ejector hooks are similar in length in *Dumontia* and there is a neighboring seta (Fig. 2A).

(B) Constituting lobes: all anomopods are invariably endowed with five pseudo-segments: three endites (E1-E3) that are largely merged with the corm, and endite 4 (E4, the IDL) and the exopodite (ODL), that have remained well individualized, even if no true segmentation is present (Dumont & Silva-Briano, 1998). Variation is observed in the demarcation and armature of the individual lobes.

Non-radopodids: Bosminidae and Ilyocryptidae: lobes highly fused, so that only 3 of them are discernible: 1 exopodite and 2 endopodites. Daphniidae with 5 lobes; variable for other groups.

Dumontiidae: the IDL has 3 setae, while the ODL has 2 setae, one claw-like. Claw-like setae are seen on the IDL of other cladoceran groups.

Radopoda: 5 lobes; variable for other groups.

Remarks: Limb 1 is variable among anomopods, and Dumontiidae has a general structure intermediate between Daphniidae and Radopoda.

(C) Size and setulation of endites 1–4:

Non-radopodids: Daphniidae: inner distal lobe (IDL) larger than outer distal lobe (ODL), and all setae are long and plumose (= covered with long setules).

Dumontiidae: the setae on the endites 1-3, and the endite 4 (= inner distal lobe), are all plumose and long. In front of the main row of endite setae, close to the inner margin, an additional seta is found, which is not modified into a Fryer's fork (*sensu* Smirnov, 1992).

Radopoda: IDL and ODL of similar shape and size. In Macrothricidae and Chydoridae, which constitutes one of the characters upon which the Radopoda is based (see Dumont & Silva-Briano, 1998), the setae of endites 1–3 differ in size and shape, and are never long and plumose. Those of endite 3, in particular, are often shorter and stouter than the others. In front of the main row of setae on the basal endites, close to the internal margin, an additional seta is found, which may be absent or modified into particular shapes in the different families. In typical Macrothricidae *sensu* Dumont & Silva-Briano (1998), for which they constitute a major diagnostic character, these setae have become transformed into a bident or trident tooth, termed 'Fryer's fork' by Smirnov (1992).

Remarks: The shape and arrangement of the setae in Dumontiidae is closer to that of the non-radopodid Daphniidae.

D. Gnathobase. Most variation on limb 1 in Anomopoda is found in this structure.

Non-radopodids: These genera typically do not have a distinct gnathobase.

Dumontiidae: There is no discernible gnathobase except for a plumose seta.

Radopoda: All members of Chydoridae and Macrothricidae have a distnict gnathobase of variable complexity.

Remarks: Dumontiidae is more similar to the non-radopodid genera in not having a distinct gnathobase lobe on this limb.

Trunk limb 2.

A. Setae on endites (building blocks of the endopodite):

Non-radopodids: Members of Ilyocryptidae have a row of 6 plumose setae, while Daphniidae have up to 8 setae, often arranged in 2 rows. Bosminidae: 2 setae on exopodite, and a variable number of setae in two series on endopodite (usually in a 6 + 5 or 6 + 2 fashion); penultimate seta modified into a scraper.

Dumontiidae: There are nine plumose setae as follow: endite 1 with 3, endite 2 with 2, endite 3 with 3, endite 4 with 2, and endite 5 with 5, and a short bluntly-rounded seta between endites 3 and 4.

Radopoda: Variable; usually 8 setae in a row.

Remarks. No clear affinities can be traced between groups based solely on the number of setae on the endites. However, the *Dumontia* limb 2 is similar to limb 1, which is more characteristic of the radopodids than the daphniids.

B. Gnathobase: in many anomopods is usually a plate, lined internally by one or two rows of spiny setae.

Non-radopodids: Bosminidae: several modified setae; with a filtering comb with doublings of setae. Daphniidae: several modified setae; with a filtering fan of about a dozen specialized setae.

Dumontiidae: The gnathobase is represented by a basal lobe with 6 non-spiny setae on endite 4.

Radopoda: Similar to Bosminidae. Filtering fan with doublings; 6–7 setae in 'non-Macrothricinae' (*sensu* Dumont & Silva-Briano, 1998), while 4–6 setae in Macrothricinae *s.str.*

Remarks. The Dumontiidae gnathobase resembles that of Radopoda genera more than the specialized gnathobase of the Daphniidae.

C. Endite near gnathobase:

Non-radopodid: variable number of setae, in one or two rows, and never modified into thick scrapers, except in Bosminidae and Dumontiidae where there is one-scraper like seta. Bosminidae: setae implanted in two rows, which in *Bosmina* amount to six anterior, and five posterior setae (Kotov, 1995), while in *Bosminopsis* there are six anterior and two posterior setae (Kotov, 1997). The penultimate seta (counting from outwards to inwards) is modified into a scraper. Daphniidae: all setae are long and plumose; none of them scraper-like as in Radopoda.

Dumontiidae: There are 4 apical setae, all different in length and shape, and 6 plumed setae on the filtering fan on gnathobase. 'Scraper-like' seta is the penultimate. This seta is different from the typical radopodid and bosminid scrapers. Setae on endites arranged in a 3-2-3-2-5 fashion (Fig. 2B).

Radopoda: A continuous line of 7-(8)-9 scraping setae on endite.

Remarks: The setae on this endite in Dumontiidae are closer in shape to those in non-radopodids.

Trunk Limb 3.

In Anomopoda, this limb is composed of a single-lobed exopodite, provided with 6 plumose setae, and of a more complex endopodite, again resulting from a degree of fusion of a corm with a series of endites. The exopodite is interpreted as composed by more than one unit in some groups.

A. General structure:

Non-radopodids: Ilyocryptidae: only one endite and 2 exopodite units are discernible; outer lobe with a spine and a filter comb. The endopodite can be seen as further fused, and only an external and an internal lobe have remained individually recognizable. In Daphniidae there are four endites, 3 carrying terminal setae and a gnathobasic comb with variable number of highly-specialized filter setae.

Dumontiidae: Four endites remain visible plus a posterior filter comb made up by 9 long setae, 2 of them spine-like and one unaligned with the rest. There are three lobe-like fused endites each with 2 similarly long plumed setae, accounting for the six long setae projecting from median part.

Radopoda: Similar to Ilyocryptidae.

Remarks: The general shape of limb 3 in Dumontiidae is closer to that of the non-radopodid Daphniidae, except that the *D. oregonensis* has only a few rather disorganized filter setae.

B. Internal lobe:

Non-radopodids: Ilyocryptidae: armed with a marginal row of plumose setae. Several genera have modified setae and receptor organs

Dumontiidae: The lobe has a row of marginal plumose setae like in the Ilyocryptidae, but lacks the short, often found in the Radopoda. *Dumontia* has a row of 4 setae on inner face, besides the marginal row of 4 short-setae.

Radopoda: Marginal row of setae composed by short, often modified setae and receptor organs.

Remarks: The armature of the internal lobe in Dumontiidae is similar to that observed on the non-radopodid Ilyocryptidae.

C. Gnathobase:

Non-radopodids: Bosminidae: no distinction between inner and external lobes; setae on inner lobe absent; complex filter comb often present in these families.

Dumontiidae: Complex filtering screens are absent. It is fused to main corm and carries three setae in *Dumontia*. The largest of the setae is a long, unaligned, plumose seta, and one of the other 2 setae is a thorn-like, crooked seta.

Radopoda: Largely fused to main corm; armature of inner lobe variable; filter comb-well developed. Is diagnostic for Radopoda in that the gnathobase has become fused to the internal endite. A crooked seta of variable length and strength is typical in Chydoridae and most Macrothricidae (Dumont & Silva-Briano, 1998).

Remarks: The gnathobase of limb 3 in Dumontiidae seems intermediate between those of non-radopodids and Radopoda.

Trunk limb 4.

A. General arrangement:

Non-radopodids: In Ilyocryptidae the exopodite is large and the endopodite is composed of a gnathobase with terminal armature and a small filter comb; rest of endopodite with ca. 14 unaligned setae. In Daphnidae, limb 4 is very similar to limb 3. In Bosminidae, the endite is specialized, armed with 4 comb-like setae. According to Dumont & Silva-Briano (1998), these setae are analogous to the 'burning-torch' setae of Radopoda.

Dumontiidae: Limb 4 is similar to limb 3. In *Dumontia* there is a comb of 5 plumed setae, the gnathobase is fully fused to the endite and simple with only 7 loosely-arranged filter setae (not like the tightly-packed filter setae in Daphnidae); and there is no suggestion of a 'burning-torch' structure like in Radopoda.

Radopoda: Variable; exopodite from large to extremely small and armed with 2–8 plumose setae; endopodite with a marginal row of 5 setae variously modified (from 'burning-torch' to barrel-shape). A posterior filter comb is present or absent.

Gnathobase:

Non-radopodid genera and **Dumontiidae**: None of the gnathobase setules resembles the radapodid 'burning-torch' setae.

Radopoda: The gnathobase is even more fully absorbed into the endite than on limb 3, and occasionally vestigial. The typical marginal arrangement of the endite consists of an external spine, three modified setae (often barrel-shaped, and crowned by 'flaming-torch' shaped setules) (Dumont & Silva-Briano, 1998).

Remarks: Gnathobase of limb 4 in Dumontiidae is considerably reduced and unlike that in Radopoda. Further studies on this structure are required for other non-radopodid groups.

Trunk limb 5.

A. General structure: There appears to be ambiguity in the interpretation of the fifth limb morphology between radopodids and non-radopodids, as to whether the inner lobe is a gnathobase or an endopodite.

Non-radopodids: Diverse morphology (Dumont and Negrea, 2002 Fig. 33) includes: Ilyocryptidae: exopodite large with 7 plumose setae; endopodite with 2 setae. Bosminidae: exopodite large with 5 setae; endopodite without long plumed setae. Daphniidae: entire limb with four setae (Alonso, 1996 Fig. 58).

Dumontiidae: The limb (Fig. 3A) The limb exopodite composed of a segment with two plumose setae; the endopodite has a single seta, and there is no discernible gnathobase.

Radopoda: Variable; in some groups the endopodite and gnathobase are thought to be fused into a single structure as in *Bunops* (Silva-Briano, 1998). *Bunops* also appears to have a two-segmented exopodite.

Remarks: The structure of limb 5 in Dumontiidae (with a large epipodite, only three plumed setae and a simple inner endopodite or gnathobase) is similar to that of the non-radopodid Daphniidae. Limb 5 of have several additional setae, a smaller epipodite, and a larger gnathobase which often has a filter comb.

B. Gnathobase:

Non-radopodids: Ilyocryptidae: apically pointed, with 2 setae and a small filter comb. Bosminidae: well-developed, with 4 apical elements and no filter comb. Daphniidae: no trace of a gnathobase or represented by a small lobe.

Dumontiidae: There is no trace of a gnathobase.

Radopoda: The gnathobase is fused to the corm.

Remarks: The expression of a gnathobase on limb 5 is variable among members of the Anomopoda; it is vestigial in Dumontiidae and other non-radopodids, but also vestigial or absent in Radopoda.

Trunk limb 6.

Non-radopodids: Ilyocryptidae: a small lobe. Daphniidae: absent; daphniids only have 5 pairs of trunk limbs. *Bosmina* has a finger-like projection without setae or setules (Dumont & Negrea, 2002 Fig. 50).

Dumontiidae: a lobe with setules but no plumed setae. There appears to be an inner lobe with two projections.

Radopoda: if present, a single lobe without plumed setae, and one or more smaller inner lobes (Dumont and Negrea, 2002 Fig. 50).

Remarks: The *Dumontia* limb 6 resembles that of radopodid genera.

Dumontia *n. gen.*

Material examined: This is a monotypic genus, and the material examined is the same as for the family and genus. The morphological description is given under the description of the new family Dumontiidae.

Dumontia oregonensis *n. sp.*

Etymology. The species name, *oregonensis*, refers to the state of Oregon, U.S.A.

Material examined: This species is in a monotypic genus and family, and the material examined is the same as for the family and genus. The morphological description is given under the description of the new family Dumontiidae.

Dumontia as a rare species

Previous unpublished records on the zooplankton diversity of the Agate Dessert ephemeral pools refer to *Dumontia oregonensis* as *Macrothrix* sp. (Borgias, pers. com.). *Dumontia* was collected over the period from January 23 through April 5, 1998, from the Agate Desert Preserve, Whetstone Savanna Preserve, and the Oregon Department of Wildlife- Denman Wildlife Area (southern tract), all on the Agate Desert landform. It was also collected during the same period in one pool on Lower Table Rock, very nearby, but up 800 feet in elevation on an andesitic lava outcrop with vernally wet flats and pools. All of the sites are within about 2 miles of each other.

The Agate Desert Preserve contains over 80 small pools, of which only about 40 pools fill with water each year during the early winter and then dry in late winter. *Dumontia oregonensis* was found in 2 of 5 pools sampled in 1998, but these 2 pools were still dry in March, 2001, a drought year (Borgias, pers. comm.). The species was found in only one out of 16 ponds sampled in 2001, pool #7 named by the Nature Conservancy of Oregon.

At Whetstone Savanna, also in Oregon, roughly 90 pools typically form in wet years, but only three pools had any water by the dry, late winter of 2001. None of these were checked for *Dumontia* in 1998, and none of the samples checked in 2001 contained specimens of *Dumontia*. The number of studied samples is too small to rule out other areas in Oregon as possible localities for *D. oregonensis*, but so far the species is known only from a very restricted area.

Although preliminary, our observations suggest that *D. oregonensis* occupies a particular habitat very limited in time and space. Considering the shallow and ephemeral nature of its habitat, it is remarkable that we did not find any ephippial females.

Very little is known about the biology of this novel cladoceran in the wild. However, we noticed that the single sample containing this species lacked individuals of a large species of *Simocephalus*. *Simocephalus* sp. was present in 8 of the 13 samples received from The Nature Conservancy. *Simocephalus* sp. outcompeted *D. oregonensis* when both species were reared together in the laboratory conditions.

Dumontia classification and the order Anomopoda

In line with the scheme proposed by Dumont & Silva-Briano (1998), the most primitive Radopoda have the most complex, almost ctenopod-like limbs, and a limb 6 should only be expected in these primitive forms. Indeed, only four genera, the non-Macrothricinae *sensu* Dumont & Silva Briano (1998) were previously known to have a rudimentary limb 6, and only in *Ophryoxus* and *Parophryoxus* does this limb have a slightly lobular structure. Limb 6 in *Dumontia*, which, as in *Eurycercus* and *Acantholeberis*, is a simple, setulated lobe with a vestigial epipodite. In *Eurycercus*, the setulation is unilateral and covering the whole margin, while in *Acantholeberis* the setulation is more or less apical. In this regard, *Dumontia* has an intermediate limb 6, which is unilaterally and apically setulated, with longer setules in apical position.

Dumont & Silva-Briano (1998) visualized the ancestral radopodid cladoceran as a rather large anomopod, without a rostrum, with no or an imperforated dorsal head pore (a window), a second antenna with a three and a four segmented branch, an incomplete limb 6, rather large filter combs on the endites and gnathobases of limbs 3–5, and the endite of limb 2 with two rows of differently structured setae, the anterior one having become more strongly developed and specialized for scraping or raking food particles.

Dumontia differs from the ancestral anomopod of Dumont & Silva Briano by having a small but distinct rostrum and gnathobases of limbs 4–5 that are not differentiated in *Dumontia*. Another character suggested for the ancestral prototype that is not seen in *Dumontia* is the presence on limb 2 of a line of 8 (plus or minus one) scraper-like setae lining the endite rim.

Dumontia could be an ancestral anomopod early on its way to becoming a 'radopodid' cladoceran, whose limbs still retain a morphology typical of daphniid cladocerans, although the filtering fans are poorly developed in *Dumontia*. The opposite situation would be also possible: that *Dumontia* is an advanced anomopod that evolved from a 'radopodid' ancestor and acquired a filtering limb morphology better adapted to invade the limnetic zone and, therefore, lost the scraper-setae of typical radopodids.

Dumont & Silva-Briano (1998) considered *Neothrix* to be so different from true Macrothricidae that it deserved family status. *Neothrix* lacks two structures diagnostic of the Anomopoda: ejector hooks on limb 1, and end-claws on the postabdomen (females).

Dumontia is even more different from the macrothricids than is *Neothrix*. Although *Dumontia* resembles the macrothricids externally, there are many differences in setation and limb structure. The setae along the ventral margin of the carapace are bifurcated in *Dumontia*, as seen in Ilyocryptidae, but never so in Macrothricidae. The major differences between Dumontiidae and Macrothricidae reside in the trunk limbs. These limbs in Dumontiidae seem better suited for filter-feeding, while Macrothricidae have limbs typical of benthic animals, and which are more adapted to scrape on surfaces.

Smirnov (1992) proposed the removal of the Ilyocryptidae from the Macrothricidae, two families that only present some external convergence. On evidence of the trunk limb structure, Dumont & Silva-Briano (1998) supported this decision and indicated that Ilyocryptidae is perhaps remotely related to the Bosminidae. The trunk limbs of Ilyocryptidae differ from Macrothricidae to a similar extent as do the limbs of *Dumontia* from Macrothricidae. The limb morphology of Dumontiidae is closest to that of Daphniidae, but the antennules, postabdomen, valves and head of *Dumontia* are quite different from those of *Daphnia*. Because of these morphological comparisons, we propose that *Dumontia* is sufficiently different to be granted the status of a new anomopod family: the Dumontiidae.

The question remains as to whether the family Dumontiidae should be placed within an emended suborder Radopoda. If so, the lack of radopodid scrapers on limb 2 could no longer be considered as an exclusion criterion of the Radopoda. Alternatively, although the gross body anatomy is radopodid-like, the resemblance in limb morphology with daphniids suggests that Dumontiidae belongs in a non-radopodid taxon within the Order Anomopoda. On the basis of the differences in limb morphology detected between the *Dumontia* and the Radopoda, and the closeness in the same limb morphology between *Dumontia* and Daphniidae, we consider *Dumontia* as a non-radopodid taxon. Until more evidence is available, we recommend retaining the suborder Radopoda.

We conclude that Dumontiidae represents a new family of anomopods, with limb morphology as seen in non-radopodid cladocerans, but with a body that superficially resembles the members of the radopodid Macrothricidae. The trunk limbs of *Dumontia* and *Macrothrix* are remarkably different, but they both have similar antennules, natatory antennae and the head fused to the rest of the body. *Dumontia* and *Macrothrix* present a system for future studies of possible convergences in cladocerans.

Dumontia resembles in most respects the hypothetical stem anomopod proposed by Fryer (1995). The general body forms are quite similar. Like the hypothetical anomopod, *Dumontia* has only a few simple filtering setae on the trunk limbs, six trunk limbs, a rostrum, and a prominent first antenna attached to the anterior tip of the head. The *Dumontia* swimming antenna has nine swimming setae on the second antennae, (the stem anomopod shows 10). Like the proposed stem, *Dumontia* reproduces parthenogenetically, and *Dumontia* probably retains the ancestral association with the bottom and vegetation.

Unlike the hypothetical anomopod but like most modern anomopods, *Dumontia* has an unsegmented first antenna, (similar to Daphniidae and Moinidae). Also, the fifth trunk limb resembles that of Daphniidae, not the relatively simple fifth limb of the proposed stem anomopod.

This new species and family provides a powerful tool for understanding evolution and phylogeny of anomopods. We look forward to seeing the morphology of the male and the resting egg, and we encourage cladistic and ecological studies of *D. oregonensis*.

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Fish zonations and guilds as the basis for assessment of ecological integrity of large rivers

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Abstract

Longitudinal zonation concepts describe the downstream changes in chemico-physical and biological properties of rivers. Including information on ecological fish guilds can enhance the usefulness of fish zonation concepts, in a way that they can be used as tools for assessment and management of the ecological integrity of large rivers. We present an ecological characterization of fish zones and fish communities in near-natural and in regulated large rivers in Europe (the River Doubs in France and the Rivers Rhine and Meuse in the Netherlands), using guild classifications of several life-history traits of fish and national Red Lists of threatened species. The Doubs data set was also analyzed using indices of the sensitivity of fish species to environmental degradation and indices for eurytopy. In these rivers, the number of ecological guilds per zone increases downstream, and there are clear shifts in the structure of the guilds. Flow preference and reproduction ecology of river fish are closely linked. The proportion of rheophilic species in the fish community decreases downstream, and the proportions of limnophilic and eurytopic species increase. Lithophilic and psammophilic spawners are dominant in the upper zones, whereas the lower zones are dominated by phytophilic and phytolithophilic spawners. The proportion of zoobenthivorous and periphytivorous species decreases downstream, and the proportion of zooplanktivorous and phytivorous species increases. However, because the European fish fauna mainly consists of feeding generalists, the discriminative abilities of simplistic feeding guild classifications are not very high. Guilds of sensitive, stenoecious species that share life history strategies that are highly adapted to specific riverine conditions (rheophils and limnophils) have declined far more than generalist species that can survive in a wide range of habitats that are not characteristic of natural river ecosystems. Because of the subsequent over-abundance of the eurytopic species the original longitudinal fish zonations are hardly recognizable anymore in heavily impacted large rivers such as the River Rhine. Hence these rivers do not meet the criteria for ecological integrity. Within a specific fish region, a suitable way of analyzing and monitoring the impact of human disturbance on the structure of the fish community is by comparing the guild structure of the present state of a fish zone with that of the reference situation.

Introduction

The physical, chemical and biological characteristics of every natural large river change gradually along its course from headwaters to mouth. One conspicuous result of these shifting environmental properties is that most fish species find suitable living conditions in only a selected stretch of the entire river. Already in the 19th century ichthyologists used this observation as the basis of a zonation system for river courses, in which the entire river was divided into separate zones, each with its own characteristic fish species (Holčík, 1989). Various fish zonation concepts have been proposed, and many have been subsequently adjusted or refined. Much effort was put into describing the physico-chemical and biological properties of the fish zones, in order to try to enhance the predictive abilities of the zonation concepts. This paper contains a comprehensive summary of zonation concepts that are most applicable on European large rivers, with emphasis on the ecological characteristics of the zones. The structure of the fish communities of the fish zones in a river in its original, natural state can be an important reference for the assessment and restoration of the ecological integrity of that same river in its present disturbed state (Lenders et al., 1998; Schmutz et al., 2000). Knowledge about original fish zonation structures can also be applied in the assessment of the ecological integrity of present-day rivers, as prescribed by the European Water Framework Directive (EC, 2000; EC-DG XI, 2003): e.g. to what extent do European large rivers still show (original) zonation patterns (Van der Velde & Van den Brink, 1994; Chovanec et al., 2000; Schmutz et al., 2000).

Longitudinal zonation concepts describe the changes in environmental properties of river courses, but they do not explain them. Another drawback of zonation concepts is that they seem to apply rigid, abrupt boundaries between zones, which does not correspond to the gradual transitions shown by real rivers. The River Continuum Concept (RCC; Vannote et al., 1980) does not have these drawbacks, and it is generally regarded to be a more realistic model of river structure and functioning (Bayley & Li, 1992; Lorenz et al., 1997). The RCC emphasizes the gradual nature of the changes that occur in biological processes in rivers, and the ways in which biological communities react to these changes. However, although the RCC does not apply to discretely defined river zones, it does not reject the sequence of changes in ecological properties that already emerged from longitudinal zonation concepts either; it explains and elaborates them. Therefore, fish zonation concepts retain much of their value, because they can specify rather precisely how fish communities react to changing river properties. Fish zonation concepts are widely used in contemporary river fish studies and form an integral part of the scientific field of river ecology.

To enhance the feasibility of fish zonation concepts as assessment tools, it is crucial that fish data are analyzed in more sophisticated ways than just recording the presence or absence of species (which used to be common practice in many fish zonation studies). Fish data will have to be interpreted ecologically, so they yield information about riverine habitats and processes. One way in which this can be achieved is to distinguish functional groups (or guilds): groups of species that exploit a resource (food or habitat) in a similar fashion, and that can take over each other's functional roles in an ecosystem (Bain et al., 1988; Bergers, 1991). Species can be grouped into guilds on the basis of many different life-history traits, and an important aim of this study was to investigate which guild classifications that have been proposed in the ichthyological literature are suitable for ecological analysis of fish zone data. In the second part of this paper, some suitable fish guild classifications will be used to analyze the natural fish zonation of two well-described river systems, the Rivers Rhine and Meuse in the Netherlands and the River Doubs in France, in more or less undisturbed states.

For an indication of the present state of fish zones and fish guilds in disturbed large rivers, national Red Lists of threatened and vulnerable fish species can be used. A Red List is an inventory of the current conservation status of plant and animal species; it uses a set of objective, scientifically-based criteria to evaluate the relative risk of extinction of species. Because the reasons for the decline of many fish species on Red Lists are known (Kirchhofer & Hefti, 1996; De Nie, 1997; De Nie & Van Ommering, 1998), the presence or absence of these species can give an indication of the most important human impacts that have affected a certain fish zone or fish guild. Fish species can also be classified a priori as 'sensitive' or 'tolerant' with regards to susceptibility to physical and chemical degradation of their habitats. Including this information can enhance the feasibility of fish assessments as tools for river management.

Ecological integrity for streams implies the presence of an adaptive assemblage of organisms having a species composition, species richness, and functional organization comparable to that of natural habitat in the region (Karr, 1995). The aims of this study are: (1) to give an ecological characterization of fish zones and fish communities in near-natural and in regulated large rivers in Europe, using fish guild classifications and Red Lists of threatened and vulnerable species, and (2) to assess the ecological integrity of these rivers.

Materials and methods

Longitudinal zonation concepts for large rivers

We searched the ecological literature to distill a comprehensive picture of the fish zonation concepts that have been drawn up for large rivers, especially those that are applicable to western Europe. An overview of these fish zonation concepts and the ecological properties of the zones is presented in the Results section.

Ecological fish guilds

Abiotic factors define the fundamental niche of every species. Biotic interactions (competition and predation) reduce this fundamental niche to the realized niche, in which the species actually exists (Quak, 1994). In ecological studies fish species sharing more or less the same niche are often grouped into guilds (functional groups) of species that exploit a resource (food or habitat) in a similar fashion (Bain et al., 1988; Bergers, 1991). The guild structure of an ecosystem is often more stable in time than its species composition, because the species within a guild can 'take over' each other's functional role, following slight fluctuations in environmental conditions. Studying the distribution of guilds in space and time can give distinctly different information than studying the presence of species (Simberloff & Dayan, 1991), and has yielded some of the most important theories in ecology (for instance biodiversity-stability hypotheses (Aarts & Nienhuis, 1999) and the River Continuum Concept (Vannote et al., 1980)).

Species can be grouped into guilds on the basis of many different life-history traits. We searched the ichthyological literature for fish guild classifications that are suitable for ecological analysis of fish zone data. In the Results section some current guild concepts related to feeding, habitat use, and reproduction are presented, and used to assess the underlying causes and ecological mechanisms of the depauperate state of the present ichthyofauna of most large rivers in Europe.

Data can be processed in different ways to express the guild structure of a fish community. The abundance of individuals of each species is a good measure, and often used for this purpose. An alternative measure, biomass per species, is also used widely, but can give a completely different picture. In this study, the number of species per guild is used as a crude estimator of guild structure, because available data (especially historical data) are often not detailed enough to analyze abundance or biomass patterns, and because comparison of different studies is often hampered by different fishing techniques.

River fish data sets

Although there has been, for a while, quite a lot of scientific interest in river zonation concepts, published data sets describing the occurrence of *all* fish species in *all* zones of medium-sized or large rivers are rather scarce. We found two suitable data sets that are comprehensive enough to enable ecological analysis of fish guild structures. Siepel et al. (1993) provided a list of all 47 fish species occurring in the various fish zones in the Rivers Rhine and Meuse. Although this data set is usable, it is more or less theoretical and it is certainly historical, because it is based on expert judgement about the zones in which the fish species occured in the Rivers Rhine and Meuse when these were still in a natural state. To verify the ecological patterns that were inferred from it, we searched for empirical data on fish distributions in moderately impacted river systems in Europe. A suitable data set was provided by Verneaux (1973), who investigated the macrofauna and fish assemblages of the River Doubs in northwestern France. The River Doubs is a medium-sized, sixth order river in the Rhône-Saone catchment, and includes Trout, Grayling, Barbel and Bream zones (but no anadromous species). At the time of investigation the river was still quasi-natural with no channelization and little regulation. Water pollution was only moderate and mainly local.

Red Lists and sensitivity to environmental degradation

To analyze the present ecological values of fish species, ecological guilds and zones, we incorporated the status of the species on the national Red Lists of threatened and vulnerable freshwater fish (Keith, 1994; De Nie & Van Ommering, 1998). However, the French Red List contains only few species compared to those of the Netherlands, and therefore it does not give a clear indication of the present status of ecological guilds or fish zones. To make comparison of the fish data from the Doubs study with the Rhine-Meuse study more meaningful, the status of fish species from the Doubs river on the stricter European Red List (Lelek, 1987) was used.

The status of a species on Red Lists could be seen as an indication of the sensitivity of the species to environmental degradation, analogous to the methodology used in 'Index of Biotic Integrity' (IBI) classifications for labelling species as 'tolerant' or 'sensitive' on the basis of population trends (Karr, 1981; Simon, 1999). For French freshwater fishes some alternative sensitivity measures are available. For each species in the River Doubs study Verneaux (1973, 1981) calculated a resistance index Ir ('indice de résistance à la dégradation du milieu'). A fish species with a low Ir is very sensitive to perturbations of its habitat, whereas a species with a high Ir could be called tolerant or resistant against perturbations. For several species Verneaux (1973) also calculated an index for the sensitivity to pollution (Ip). Grandmottet (1983) analyzed the microhabitat use for reproduction, feeding and shelter of French freshwater fishes. Each species is characterized by an index of eurytopy E, which indicates whether a species can reproduce, feed or find shelter in many or in only a few different microhabitats (euryoecious versus stenoecious species). Per species and per function (reproduction, feeding and shelter) subindices are calculated seperately for lotic and lentic microhabitats (Ec and El). We used these indices as an additional means to assess the sensitivity of the fish guilds and zones in the Doubs data set. Because these index values are not available for many species in the Rhine-Meuse data set, these sensitivity indices could not be used for analysis of that data set.

Results: ichthyological analysis of longitudinal zonation patterns in large rivers

Longitudinal zonation concepts for large rivers

Fish zones

Already in the 19th century eastern European ichthyologists had drawn up a rough classification system for the longitudinal succession of characteristic or dominant fish species that occur in rivers (Fritsch, 1872; Von dem Borne, 1878; Nowicki, 1889; in: Holčík, 1989). They divided the entire course of a river, from the spring to the sea, into 5 basic zones: Trout (*Salmo trutta*), Grayling (*Thymallus thymallus*), Barbel (*Barbus barbus*), Bream (*Abramis brama*) and Smelt (*Osmerus eperlanus*) zone. Huet (1949, 1959, 1962) improved this classic scheme by determining the characteristic physical and chemical parameters of each zone: the slope, the width, the depth, the current velocity and the water temperature (Lelek, 1987; Holčík, 1989) (Table 1).

The following short description of the zones is based on Siepel et al. (1993) and De Nie (1998), and adds some later subdivisions and the present occurrence of the zones in the Netherlands.

1. Trout zone: narrow, shallow, fast flowing clear waters. The water is nutrient-poor and cold. The soil consists of clean gravel, sand and locally a little silt. Some authors have made further subdivisions of the Trout zone, such as Upper and Lower Trout zone. In the Netherlands only a few tributary brooks of the small river Geul (Meuse watershed) possibly belonged to the Trout zone.

2. Grayling zone: fast flowing clear waters. The water is a little richer in nutrients, the soil consists of gravel, sand and silt, and the brook is deeper and wider than the brooks in the Trout zone. In the Netherlands the small river Geul and the upstream stretches of a few brooks belong to the Grayling zone.

3. Barbel zone: wide, lotic, often clear waters. This is the middle reach of a river, running through sloping hills. The water is a little richer in nutrients than in the Grayling zone. The characteristic fish species of the Barbel zone require clean gravel for completing their life cycle. In the Netherlands only the uppermost part of the river Meuse, the so-called Grensmaas, belongs to the Barbel zone. However, the dominant species here is Chub (*Leuciscus cephalus*), not Barbel. According to Volz & Cazemier (1991) two branches of the Rhine river system, the IJssel and the Nederrijn, show some morphological characteristics of the Barbel zone.

4. Bream zone: stagnant or slowly flowing, clear or turbid waters. This is the traditional zone of the lowland river. The water is slightly nutrient-rich by nature. To the Bream zone also belong the stagnant water bodies that result from natural meandering processes and the more or less isolated stagnant water bodies in the floodplains. The Bream zone used to be very dynamic in space and time because of the ongoing ecological succession, creating a vast variety of habitats, like bare gravel bars, steep banks, sheltered waters with submerged vegetation, reed marshes and floodplain forests. Originally the fish community inhabiting this zone was very species-rich. However, in recent times eutrophication has led to an over-abundance of the Bream. Most stretches of the large rivers in the Netherlands belong to the Bream zone.

5. Smelt zone: river mouths and brackish waters. This zone is subdivided into two subzones (Schouten & Quak, 1994; De Nie, 1998): the Upper brackish water zone, called Ruffe (*Gymnocephalus cernuus*) zone,

Table 1. Longitudinal river zonation concepts: fish zones (Huet, 1949) and biocoenotic zones (Illies & Botosaneanu, 1963), with additional hydrological and ecological characteristics (Lelek, 1987; Holcik, 1989; Siepel et al., 1993)

Fish zone		Biocoenoses	River type	Order	Erosion	Current (cm/s)	Production	Fish fauna	Dominant species	Species richness	Reproductive guilds
Springs		Eucrenon	Springs	1–3	Erosional		Production	No fish		Low	
		Hypocrenon	Head streams	1–3	Erosional		Production			Low	
Trout	Upper Trout	Epirithron	Mountain brooks with waterfalls	3–6	Erosional	30–50	Production	Salmonids	Trout; Bullhead, Minnow	Low	Lithophils
	Lower Trout	Metarithron		3–6	Erosional	30–50	Production	Salmonids	Trout; Bullhead, Minnow	Low	
Grayling		Hyporithron	Upper submountain stream with braids	3–6	Intermediate	25–50	Transfer	Mixed fauna, salmonids dominant	Grayling; Trout zone species; Rheophilic cyprinids	Medium	Lithophils
Barbel		Epipotamon	Lower submountain river, anastomosed	3–6	Intermediate	10–25	Transfer	Mixed fauna, cyprinids dominant	Rheophilic cyprinids; Associated cyprinids and predators	High	(Phyto)-lithophils
Bream		Metapotamon	Lowland large river with meanders	6-	Depositional	0–10	Storage	Cyprinid fauna with predators	Limnophilic and associated cyprinids and predators	High	Phytophils
Smelt	Ruffe	Hypopotamon	Estuary	6-	Depositional		Storage	Mixed fauna, no cyprinids		Medium	Pelagophils
	Flounder	Hypopotamon		6-							

and the Lower brackish water zone, called Flounder (*Platichthys flesus*) zone. (a) the Upper brackish water zone (hyaline potamon) consists of a very dynamic landscape: the slow-flowing river deposits silt and the nutrient-rich water leads to ecological succession. Sometimes the water is brackish, so fish species that occur in this zone have to be adapted to this. This zone is important for diadromous fish. (b) the Lower brackish water zone (the estuary) is constantly influenced by the sea: the current velocities are determined by the tide, creating deep channels and sand banks. The anadromous Smelt and Twaite Shad (*Alosa fallax*) had their spawning grounds here.

Characteristic of the Smelt zone is the large variability of all abiotic factors (Tittizer & Krebs, 1996), causing this zone to be relatively species-poor by nature. The brackish water zones of the rivers Rhine and Meuse have been severely reduced by the building of dams and sluices. This also poses great problems to anadromous fish that try to migrate from the sea up the river, because they have to adapt their physiology from a saltwater environment to a freshwater environment in a very short time span.

This classical zonation of rivers according to characteristic fish species is not used very much anymore, because it has some serious shortcomings: (1) The practical usefulness and feasibility is low (Hawkes, 1975; Quak, 1994), because the zonation is based on and intended for natural rivers, which have become rare in Europe and many other parts of the world. For instance, the entire Upper and Middle Rhine belonged to the Barbel zone, and the Lower Rhine to the Bream zone. Because of the strong anthropogenic influence this description is no longer valid, and at present the fish communities in virtually all parts of the river Rhine are alike (Lelek & Köhler, 1989; Tittizer & Krebs, 1996). Anthropogenic water bodies such as ditches, canals, lakes and pools, that are dominant in the Netherlands, do not fit in the zonation concept. However, this zonation can be valuable for the drawing up of references and targets for river rehabilitation, the planning of riverine fish reserves and for the valuation of the current fish assemblage in a river (Verneaux, 1981; Lelek, 1987; Verneaux et al., 2003). (2) The fish zonation concept is based on only one life stage of fishes, namely the adult stage (Quak, 1994). The spawning and nursery habitat requirements of fish species were not included in the zonation concept. (3)

Only the longitudinal, and not the vertical, transversal spatial and temporal dimensions have been incorporated into the fish zonation concept (Holčík, 1989). (4) The species composition of fish communities in various regions and streams is different (Holčík, 1989). Some characteristic species (Grayling & Bream) occur only in northwestern Europe, so this zonation concept can not be applied to other geographical areas. For instance, the Rhine has a very inhomogeneous ichthyofauna, caused by various specific geological events, so that the abovementioned fish zonation is not entirely applicable (Lelek & Buhse, 1992). (5.) The downstream change in the fish fauna is so gradual that a clearly recognizable species type, common to or associated with the section cannot be distinguished (Holčík, 1989). The fish zonation concept is discrete, whereas the distribution and succession of fish species is not (Quak, 1994). (6) The succession of fish zones in a river does not always follow the sequence outlined above (Huet, 1962). In most cases, it is not applicable to very large rivers (like the River Rhine), because these rivers often pass through large lakes, plateaus etc. (Illies & Botosaneanu, 1963). For example, in the course of the River Rhine the transition from the salmonid zones to the cyprinid zones occurs three times (Tittizer & Krebs, 1996)

Biocoenotic zones

To overcome the dependence on the occurrence of particular fish species, Illies & Botosaneanu (1963) suggested a new classification system, in which the previous names of the fish zones had been replaced by more general biocoenotic terms (Table 1). This new biotypological classification includes all aquatic organisms, and it also applies to running waters outside Europe with different, but convergent fauna. The most important parameters in this system are the physical structure of the river bed and the range of the water temperature during the year (Lelek, 1987). Table 1 gives an overview (based on various sources) of several biocoenotic and geomorphological classification systems of rivers, together with some physical characteristics of the zones.

Some of the shortcomings of the fish zonation concept still apply to the biocoenotic zonation concept: in real rivers the boundaries between zones are usually not clearly defined, and zones overlap one another; the downstream sequence of zones may change; some zones need not occur at all, or zones may be inverted.

Guild concepts

Feeding guilds

Widely used in zoology, fish species too can be grouped into guilds according to their feeding ecology. There is no common classification system, and many authors have deviced their own system. Some authors use very simple systems, such as Allen (1969, in Allan, 1995) (invertivores, piscivores, herbivores) or Berrebi dit Thomas et al. (1998) (invertivores, omnivores, piscivores). These simple classifications are of limited value (Allan, 1995). Adding some measure of foraging habitat can be useful. Often a vertical classification is applied of where in the water column fishes obtain their food items: benthic, demersal or pelagic. In practice there are 3 categories: surface and water column, benthic, or generalized feeders (Allan, 1995). Water column species are active swimmers that typically feed on drifting and surface invertebrates or other fishes. Benthic species, for the most part, are sensitive to siltation and benthic oxygen depletion because they feed and reproduce in benthic habitats (Oberdorff & Hughes, 1992).

Other authors have constructed very elaborate systems of feeding categories, for instance Bergers (1991), who analyzed the prey species of fishes in three river habitats in three branches of the River Rhine, subdividing each fish species into three length-categories. Clustering these 27 'fish groups' yielded 13 different feeding guilds. Goldstein & Simon (1999) have defined a common guild structure for use in studies of the Index of Biotic Integrity (IBI) in North America, using 5 basic guilds (herbivores, detritivores, planktivores, invertivores and carnivores [including parasites]) and 26 modes of feeding to subdivide these guilds, and to avoid the necessity to use an 'omnivorous' (polyphagous, opportunist, generalist) guild. Their guild structure not only incorporates food types (prey items), but also foraging habits and foraging habitats.

For most studies, a system containing 6–7 groups is adequate. In this study the feeding guild classification by Van den Brink et al. (1996) was followed: parasitic, detritivorous, zoobenthivorous, zooplanktivorous, piscivorous and phytivorous (Table 2).

Flow preference guilds

In the Netherlands, one of the oldest, and most generally applied, ecological classifications of fish species is the one based on the flow preference of adult fishes (Redeke, 1941): rheophilic (some or all stages of life

Table 2. Ecological guild classifications of river fish species included in this study, grouped according to flow preference (after Quak, 1994) **Order:** Pleu = Pleuronectiformes, Perc = Perciformes, Scor = Scorpaeiformes, Gast = Gasterosteriformes, Gadi = Gadiformes, Salm = Salmoniformes, Silu = Siluriformes, Cypr = Cypriniformes, Clup = Clupeiformes, Angu = Anguilliformes, Acip = Acipenseriformes, Petr = Petromyzontiformes. **Reproductive guild:** Li = Lithophils, Ph = Phytophils, Pe = Pelagophils, Ps = Psammophils, Ar = Ariadnophils, Os = Ostracophils, Pl = Phytolithophils, Po = Polyphils (after Balon, 1984). **Feeding guild:** Par = Parasitic, Det = Detritivorous, Ben = Zoobenthivorous, Pla = Zooplanktivorous, Pis = Piscivorous, Phy = Phytivorous, Per = Periphytivorous (after Van den Brink et al. (1996) and Lelek (1987)). **Red lists:** en = endangered, vu = vulnerable, ne = not endangered, no status indicated = species not recorded in the study of this river, * = exotic species. Doubs: European Red list by Lelek (1987), adapted for the Rhone catchment (Keith, 1994); Rhine-Meuse: Dutch Red list by De Nie & Van Ommering (1998)

English name	Scientific name	Order	Reproductive guild	Feeding guild	Red list Doubs	Red list Rhine-Meuse
Rheophilic A: *All freshwater stages of life history are confined to the main river channel*						
Rheophilic A1: *Migratory (ocean – river)*						
River lamprey	*Lampetra fluviatilis*	Petr	Li	Det/Ben/Par		vu
Sea lamprey	*Petromyzon marinus*	Petr	Li	Det/Par		en
Sturgeon	*Acipenser sturio*	Acip	Li/Pe	Ben/Pis		en
Allis shad	*Alosa alosa*	Clup	Pe	Pla		en
Houting	*Coregonus oxyrinchus*	Salm	Li/Pe	Ben/Pla		en
Salmon	*Salmo salar*	Salm	Li	Pla/Ben/Pis		en
Sea trout	*Salmo trutta trutta*	Salm	Li	Ben/Pis		vu
Rheophilic A2: *Non-migratory*						
Barbel	Barbus barbus	Cypr	Li	Ben	vu	en
Chub	*Leuciscus cephalus*	Cypr	Li	Ben/Pis/Phy	ne	vu
Nase	*Chondrostoma nasus*	Cypr	Li	Per/Ben	ne*	en
Southwest European nase	*Chondrostoma toxostoma*	Cypr	Li	Per/Ben	vu	
Dace	*Leuciscus leuciscus*	Cypr	Pl	Ben/Pla	ne	vu
Brook lamprey	*Lampetra planeri*	Petr	Li	Det	en	
Stream bleak	*Alburnoides bipunctatus*	Cypr	Li	Ben/Phy	vu	vu
Brown trout	*Salmo trutta fario*	Salm	Li	Ben/Pis	vu	en
Grayling	*Thymallus thymallus*	Salm	Li	Ben/Pis	vu	
Minnow	*Phoxinus phoxinus*	Cypr	Li	Pla/Ben	vu	en
Brook trout	*Salvellinus fontinalis*	Salm	Li	Ben/Pis	ne*	
Rhone streber	*Zingel asper*	Perc	Li	Ben	en	
Stone loach	*Barbatula barbatulus*	Cypr	Ps	Ben	ne	ne
Blageon	*Leuciscus souffia agassizi*	Cypr	Li	Ben	vu	
Rheophilic B: *Some stages of life history are confined to well connected backwaters or tributaries*						
Ide	*Leuciscus idus*	Cypr	Pl	Ben/Pis/Phy	vu	
Gudgeon	*Gobio gobio*	Cypr	Ps	Ben/Det	ne	ne
Burbot	*Lota lota*	Gadi	Li/Pe	Ben/Pis	vu	en
Spined loach	*Cobitis taenia*	Cypr	Ph	Det/Ben		ne
Rheophilic C: *Some stages of life history are confined to slowly flowing brackish water (diadromous species)*						
Smelt	*Osmerus eperlanus*	Salm	Li/Pe	Pla		ne
Flounder	*Platichthys flesus*	Pleu	Pe	Ben		ne
Twaite shad	*Alosa fallax*	Clup	Pe	Pla		en
Eurytopic: *All stages of life history can occur in both lotic and lentic waters*						
Eel	*Anguilla anguilla*	Angu	Pe	Ben/Pis		vu
Perch	*Perca fluviatilis*	Perc	Pl	Pla/Ben/Pis	ne	ne
Pikeperch	*Stizostedion lucioperca*	Perc	Ph	Pla/Ben/Pis		ne*
Ruffe	*Gymnocephalus cernuus*	Perc	Pl	Ben	ne	ne
Pumpkinseed	*Lepomis gibbosus*	Perc	Po	Pla/Ben/Pis	ne*	ne*

Continued on p. 164

Table 2. Continued

English name	Scientific name	Order	Reproductive guild	Feeding guild	Red list Doubs	Red list Rhine-Meuse
Bullhead	Cottus gobio	Scor	Li	Ben	ne	ne
Three-spined stickleback	*Gasterosteus aculeatus*	Gast	Ar	Pla/Ben		ne
Pike	*Esox lucius*	Salm	Ph	Pla/Pis	vu	ne
Schelly	*Coregonus lavaretus*	Salm	Li	Ben/Pla		en
Bleak	*Alburnus alburnus*	Cypr	Po	Pla/Ben	ne	ne
Wels	*Silurus glanis*	Silu	Ph	Ben/Pis	ne	
Asp	*Aspius aspius*	Cypr	Li	Pla/Ben/Pis		ne*
Carp	*Cyprinus carpio*	Cypr	Ph	Ben/Pla/Phy		ne
Gibel carp	*Carassius auratus gibelio*	Cypr	Ph	Ben/Phy/Det		ne*
Bream	*Abramis brama*	Cypr	Po	Pla/Ben	ne	ne
Silver bream	*Blicca bjoerkna*	Cypr	Ph	Ben/Phy/Det	ne	ne
Roach	*Rutilus rutilus*	Cypr	Po	Pla/Ben/Phy	ne	ne
Limnophilic: *All stages of life history are confined to lentic waters with macrophytes*						
Bitterling	*Rhodeus sericeus amarus*	Cypr	Os	Pla/Ben/Phy	vu	vu
Rudd	*Scardinius erythrophthalmus*	Cypr	Ph	Ben/Phy	vu	ne
Crucian carp	*Carassius carassius*	Cypr	Ph	Ben/Phy/Det		ne
Tench	*Tinca tinca*	Cypr	Ph	Ben/Phy/Det	ne	ne
Weatherfish	*Misgurnus fossilis*	Cypr	Ph	Det/Ben		vu
Sunbleak	*Leucaspius delineatus*	Cypr	Ph	Pla		vu
Ten-spined stickleback	*Pungitius pungitius*	Gast	Ar	Pla/Ben		ne
Black-bass	*Micropterus salmoides*	Perc	Po	Pis/Phy/Ben	ne*	
Brown bullhead	*Ictalurus nebulosus*	Silu	Po	Ben/Phy/Pis		ne*
Black bullhead	*Ictalurus melas*	Silu	Po	Ben/Pis	ne*	

history are confined to flowing water), limnophilic (= stagnophilic) (all stages of life history are confined to lentic waters with macrophytes), eurytopic (= euryoecious) (all stages of life history can occur in both lotic and lentic waters; ‘habitat generalists’), anadromous (adults migrate upriver to spawn) and catadromous species (adults migrate to sea to spawn). Although it is widely used, even today there is no general agreement on the position of every species in the Netherlands, and in some species different populations have developed different flow preferences (for instance: Three-spined Stickleback (*Gasterosteus aculeatus*) and Smelt have migratory (rheophilic) and non-migratory (eurytopic) populations; the Bullhead (*Cottus gobio*) is rheophilic (and endangered) in small streams, but eurytopic (and non-threatened) in large rivers, where nowadays it inhabits artificial, stony habitats (such as groynes), that are totally absent in the lowland stretches of natural large rivers. In most practical applications, each species is placed in only one category.

After detailed studies of the spawning habitat requirements of Danubian fishes, Schiemer & Waidbacher (1992) modified this flow preference system by subdiving the rheophilic class into rheophilic A (all stages of life history confined to the main channel) and rheophilic B (some stages confined to backwaters or tributaries). By further subdividing the class rheophilic B according to lentic backwaters or lotic tributaries, their classification eventually comprises 5 classes.

Schouten & Quak (1994) have refined this system even further, by subdividing the class rheophilic A into A1 (anadromous, migratory species) and A2 (non-migratory species), and adding the new category rheophilic C (some stages of life history are confined to slowly flowing brackish water (diadromous species)). Their classification is the most elaborate (6 classes) and is commonly used in the Netherlands, although it is often simplified by regrouping the 4 rheophilic classes into 3 classes: obligatory rheophilic species (all stages of life history confined to lotic river habitats; equivalent to rheophilic A2), partially rheophilic species (some stages confined to running water; equivalent to rheophilic B) and fresh water-salt water rheophilic species (the diadromous species; equivalent to classes rheophilic A1 and C). In Table 2, the river fish species used in this study are classified according to the system of Schouten & Quak (1994). Note that the catadromous Eel (*Anguilla anguilla*) is eurytopic.

Reproductive guilds

Balon (1975a,b, 1981) has classified fishes according to their spawning habitats and habits. His system is now used worldwide, with only minor adjustments. Using ethological types (guarders and nonguarders), ecological groups (describing parental investment type) and substrate types as criteria, Balon recognizes 33 guilds, 14 of which have representatives among European freshwater fishes (Holčík, 1989). See Table 2 for the assignment of river fishes to Balon's reproductive guilds.

Further refinement of Balon's system is possible. For instance, Vriese et al. (1994) have elaborated Balon's system by incorporating additional spawning habitat variables (flow rate, depth, water temperature, macrophyte cover) and data on spawning season; their classification of fish species occurring in the Dutch part of the River Meuse consists of 13 reproductive associations. Because such detailed classifications are not available for French river fishes, Balon's original classification is used in this study.

Ecological characterization of fish zones in near-natural and degraded rivers

Although longitudinal zonation concepts for large rivers have their weaknesses, they do provide a general outline of the downstream changes in environmental variables and the related fish assemblage structure that occur in natural rivers. By analyzing the species assemblages of the various fish zones in terms of ecological guilds, even more inferences can be made from these concepts. We analyzed the fish zones of the Rivers Rhine and Meuse (Siepel et al., 1993) and of the River Doubs (Verneaux, 1973) on the basis of the species classified into ecological guilds, orders and Red List categories (Figs 1–5).

On the basis of the longitudinal distribution of the macrofauna and fish species, Verneaux could distinguish a succession of ten biocoenoses (labelled B0–B9) in the Doubs from the springs to the confluence with the large River Saone. Each fish species has a typological preferendum: the biocoenoses in which it is most abundant or even dominant (Verneaux et al., 2003). The correspondence of the ten biocoenoses to the fish zones of Huet (1949) and the biotypology based on geomorphological zones of Illies & Botosaneanu (1963) is not unequivocal; here we follow the improved classification of biocoenoses into geomorphological and fish zones of Verneaux (1981) (Table 3). Biocoenoses B0, B1 and B2 are labelled as Spring zone in the Doubs data set; Siepel et al. (1993) do not distinguish a Spring zone in their data set of the Rivers Rhine and Meuse. Biocoenosis B9 of the Doubs data set is special, in that it was labelled as 'hypopotamon (excluding the estuarine zones)' by Verneaux (1981). Hypopotamon normally translates as Smelt zone, but as this lowermost reach of the River Doubs is not influenced by the sea, it can not be considered a true Smelt zone, and therefore we labelled it 'Smelt' zone. This zone should not be compared directly with the true Smelt zone in the Rhine-Meuse data set of Siepel et al. (1993), because the latter does comprise estuaries. Actually, biocoenosis B9 more closely resembles the Bream zone (B8); it has the same limnophilic and eurytopic species as the Bream zone, but the species having their preferendum in B9 are different. The most striking feature of B9 is the virtual absence of rheophilic species (unlike real Smelt zones).

Taxonomic groups

The species richness per zone increases downstream, from the Trout zone to the Barbel (Doubs) and Bream (Rhine-Meuse) zone, and decreases further downstream (Fig. 1). The number of ecological guilds also increases downstream, and there are clear shifts in the structure of the guilds.

Flow preference guilds

In both the Doubs and the Rhine-Meuse the proportion of rheophilic species in the fish community decreases sharply downstream, and the proportions of limnophilic and eurytopic species increase (Fig. 2). The Trout and Grayling zones are dominated by rheophilic species that reproduce in the main channel (rheophilic A1 and A2). The presence of floodplains with stagnant lakes and the decrease in current velocities in the main channel in the Barbel and Bream zones is reflected in the increase in limnophilic and eurytopic species in these zones, and the occurrence of rheophilic B species. In the River Doubs, the Barbel and the Bream

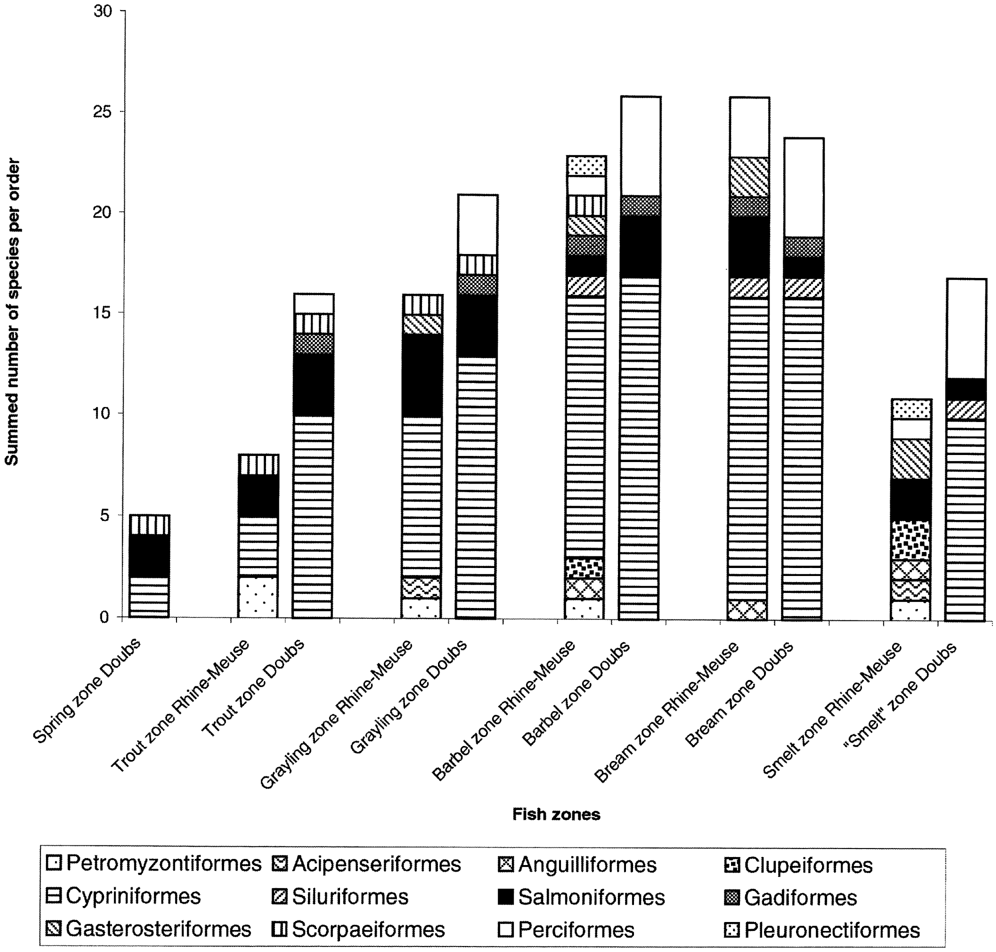

Figure 1. Taxonomic composition of fish zones of the Rivers Rhine and Meuse and of the River Doubs. The Spring zone was not distinguished in the Rhine-Meuse data set. The 'Smelt' zone in the Doubs data set does not include estuarine zones (see text for further explanation).

zones have almost the same species composition, but the rheophilic species have their typological preferendum in the Barbel zone whereas the limnophilic and eurytopic species have their preferendum in the Bream zone. Rheophilic A1 species do not reproduce in the Bream zone of the Rivers Rhine and Meuse (Fig. 2). However, the Smelt zone provides important staging areas for these anadromous species, for instance during the spawning migration when they have to adapt their physiology from salt to fresh water and vice versa. Rheophilic C species reproduce in the Smelt zone.

Reproductive guilds

Changes in flow preference and reproductive guilds are closely linked: rheophilic lithophilic and psammophilic spawners are dominant in streams and small rivers (Trout, Grayling and Barbel zones), whereas limnophilic phytophilic spawners and eurytopic phytolithophilic or polyphilic spawners predominate in lowland rivers (Bream zone) (Fig. 3). The dominance of rheophilic A1 and C species in the Smelt zone is mirrored in the abundance of lithophils and pelagophils.

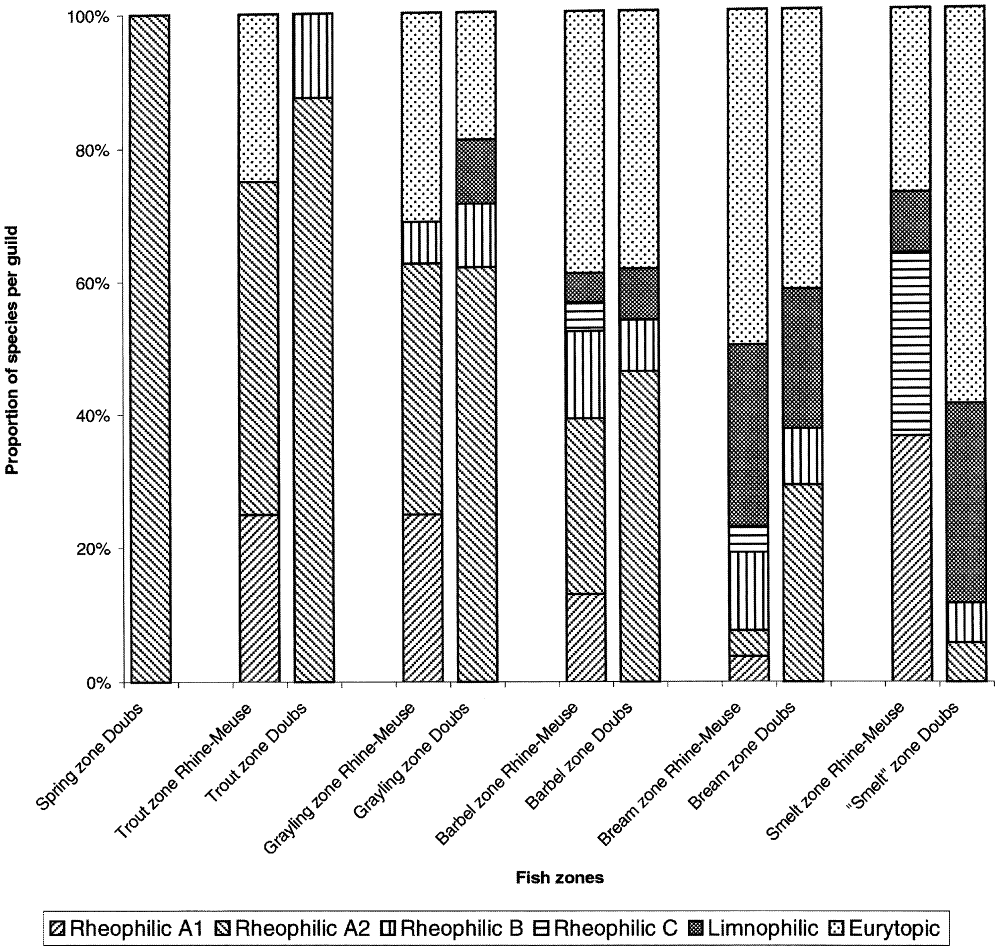

Figure 2. Composition of flow preference guilds of the fish zones of the Rivers Rhine and Meuse and of the River Doubs. The Spring zone was not distinguished in the Rhine-Meuse data set. The 'Smelt' zone in the Doubs data set does not include estuarine zones (see text for further explanation).

Feeding guilds

The downstream changes in feeding guild composition are rather slight and gradual (Fig. 4). The proportion of zoobenthivorous and periphytivorous species decreases, and the proportion of zooplanktivorous and phytivorous species increases. The proportion of detritivorous species does not show a clear trend. The percentage of piscivorous species remains fairly constant at around 15% in all zones; the proportion of parasites is based on only two species, and does not show a clear trend.

Red Lists

The present situation of the species assemblages of the various guilds and zones can be assessed using the Red List status of each species. According to the Dutch Red List, of the species used for the Rhine-Meuse assessment, 48% are not threatened, 24% are vulnerable, and 28% are endangered (Table 2). The anthropogenic disturbances to river systems and fishes have affected the various ecological guilds and fish zones unevenly (Fig. 5, Table 2). Species that are highly dependent on spawning or feeding habitats in the main river channel

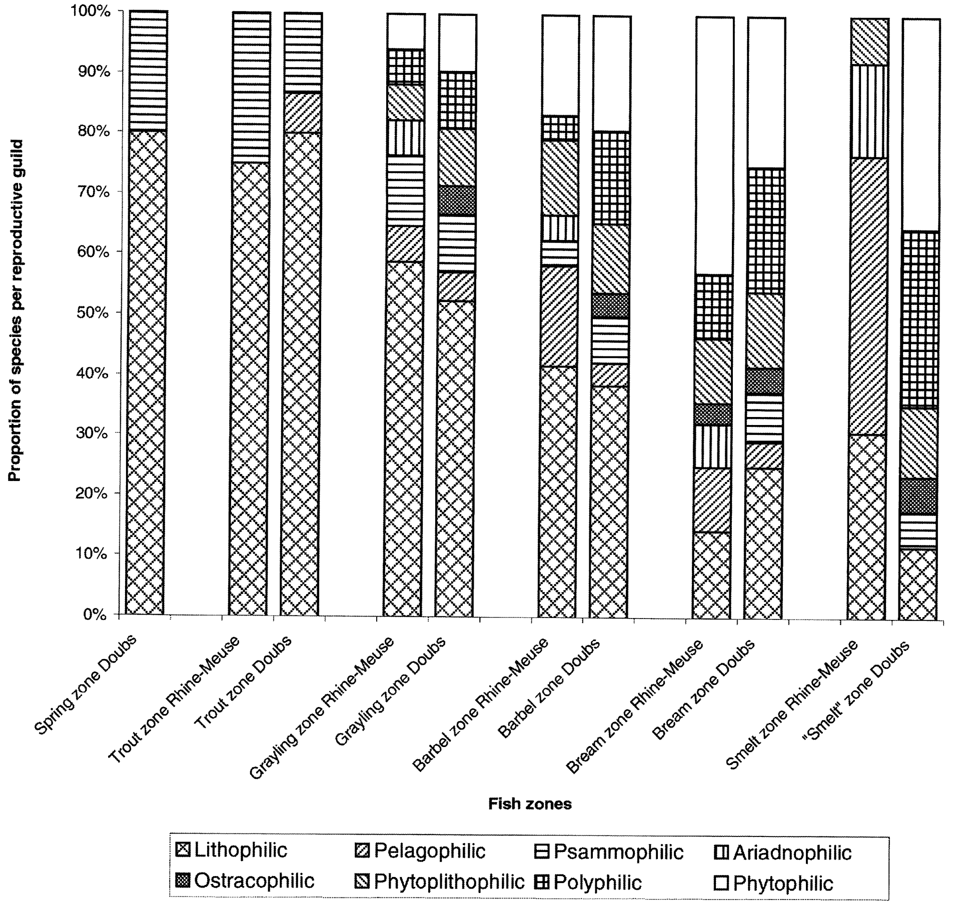

Figure 3. Composition of reproductive guilds of fish zones of the Rivers Rhine and Meuse and of the River Doubs. The Spring zone was not distinguished in the Rhine-Meuse data set. The 'Smelt' zone in the Doubs data set does not include estuarine zones (see text for further explanation).

have severely declined (Aarts et al., in press). These rheophilic species, mainly belonging to the families Acipenseridae, Clupeidae, Salmonidae and Cyprinidae, are often zoobenthivorous and pelagophilic or lithophilic spawners; 78% are on the Dutch Red List (Fig. 6). In contrast, nearly all eurytopic species are unthreatened.

Because of the differentiated distribution of the species and the guilds over the fish zones, the Trout zone has the highest percentage of Red List species, and the Bream (and 'Smelt') zone the lowest (Fig. 5). Of the Red List species in the Bream zone, the majority is susceptible or vulnerable, with only one endangered species and one extinct species there in the Rhine-Meuse data set, and none in the Doubs. The Grayling and Smelt zones in the Rhine-Meuse both have relatively high percentages of extinct species (25% and 27.3% respectively) because they used to accommodate many species from the almost extinct group of anadromous rheophils.

Indices for sensitivity and habitat flexibility

For each species in the River Doubs study Verneaux (1973, 1981) calculated a resistance index Ir. A fish species with a low Ir is very sensitive to perturbations of its habitat, whereas a species with a high

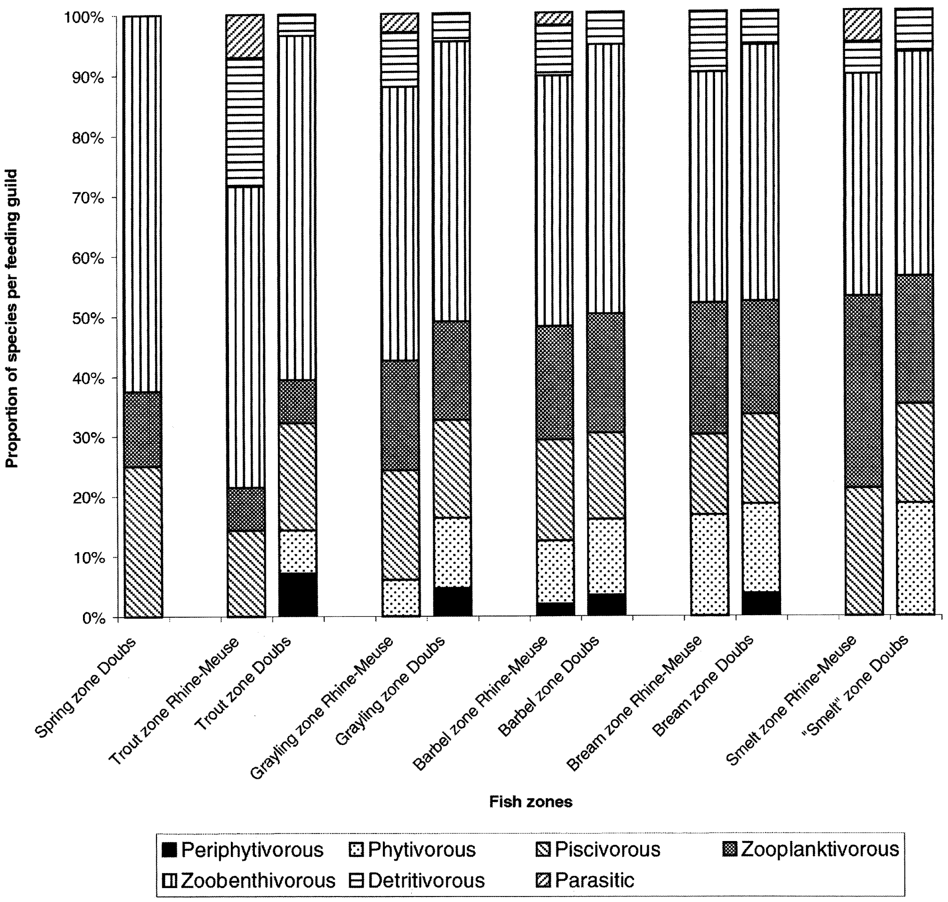

Figure 4. Composition of feeding guilds of fish zones of the Rivers Rhine and Meuse and of the River Doubs. The Spring zone was not distinguished in the Rhine-Meuse data set. The 'Smelt' zone in the Doubs data set does not include estuarine zones (see text for further explanation).

Ir could be called tolerant or resistant against perturbations. Verneaux' resistance index corresponds well to the flow preference classification (Table 3): the generalistic eurytopic species have a mean Ir of 6.72, whereas the more specialized limnophilic and rheophilic species have a mean Ir of 5.75 and 4.78, respectively.

The status of a species on the Red List could be seen as an indication of the sensitivity of the species to environmental degradation. Verneaux' resistance index also corresponds to the Red List status of indigenous species: species labelled 'not threatened' on the Dutch list have a mean Ir of 6.54, vulnerable species have a mean Ir of 5.50, endangered species 4.42 and extinct species 4.25. Species that are labelled not-endangered on the European Red List have a mean Ir of 6.25, vulnerable species have a mean Ir of 4.91 and the only endangered species (Rhône Streber *Zingel asper*) has a Ir of 3.5.

Looking at the distribution of species along the longitudinal succession of biocoenoses in the River Doubs, a clear picture emerges: the mean Ir of the fish assemblages of the biocoenoses increases downstream (Table 4). For individual species this means that generally speaking a species is more tolerant when its typological preferendum is situated further down-

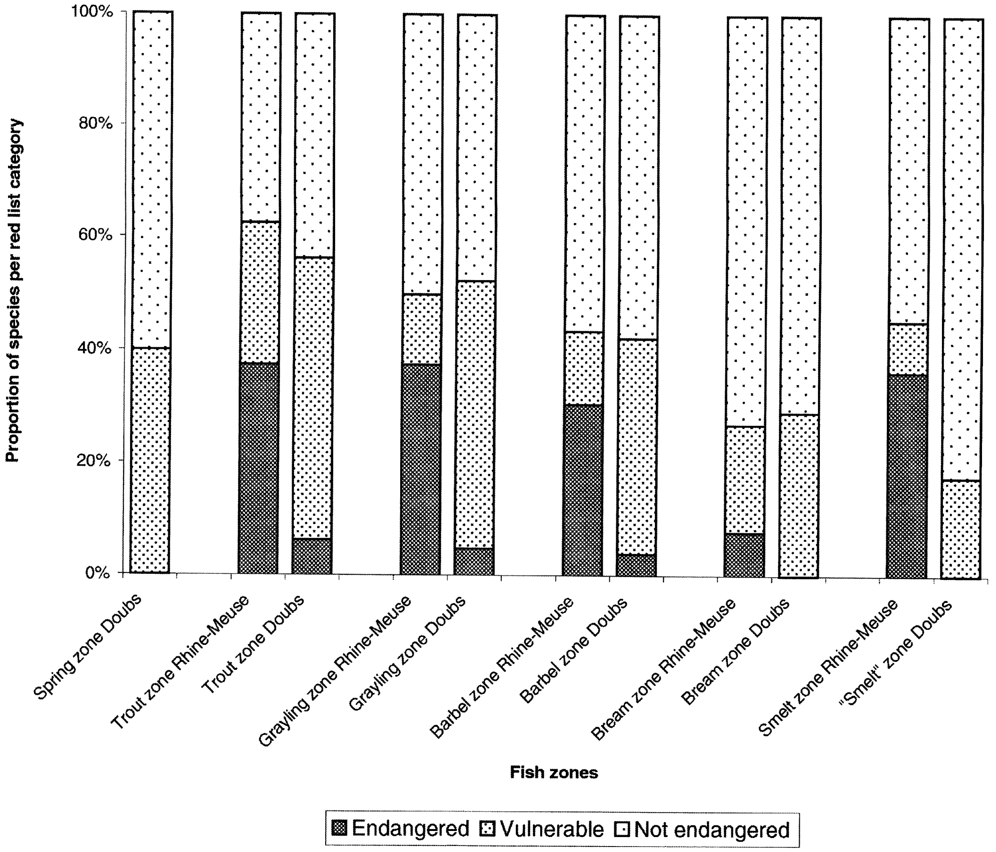

Figure 5. Red List status of fish in the fish zones of the Rivers Rhine and Meuse and of the River Doubs. The Spring zone was not distinguished in the Rhine-Meuse data set. The 'Smelt' zone in the Doubs data set does not include estuarine zones (see text for further explanation).

stream, and when it occurs in more biocoenoses (larger typological amplitude) (Verneaux, 1981; Verneaux et al., 2003). This is in accordance with the downstream decrease in the proportion of Red List species, described above. For several species Verneaux (1973) also calculated an index for the sensitivity to pollution (Ip). Roughly speaking the sensitivity to pollution increases upstream.

Grandmottet (1983) calculated eurytopy (habitat flexibility) indices for many French freshwater fishes. We applied these indices on the Doubs data set (Table 3). The indices of eurytopy increase downstream, indicating that within the rheophilic and limnophilic/eurytopic clusters the more euryoecious species occur further downstream. The subindex for reproduction (Er) of the species (the most sensitive measure) corresponds well to their flow preference classification: most rheophilic species have a lotic Er < 0.29 and limnophilic species have a lentic Er < 0.29 (both groups are thus stenoecious with regard to their reproduction substrate), while eurytopic species have a lotic or lentic Er $> = 0.29$.

Discussion: implications for habitat protection and restoration

Ecological characterization of fish zones in near-natural and degraded rivers

Siepel et al. (1993) presented a theoretical data set based on expert judgement of all fish species occurring in the various fish zones in the Rivers Rhine and

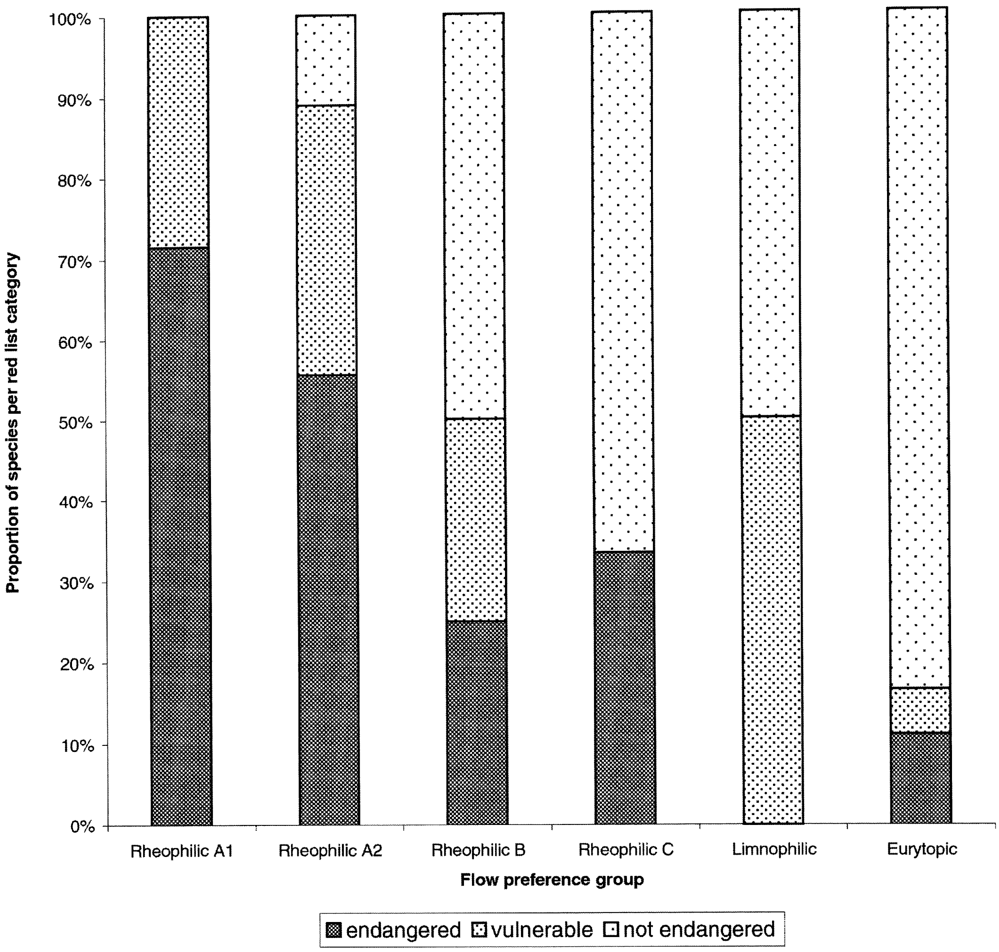

Figure 6. Red List status of flow preference groups of fish in the Rivers Rhine and Meuse.

Meuse. We used the ecological characteristics of the fish species in this data set to analyse the longitudinal zonation concepts of Huet (1949) and Illies & Botosaneanu (1963). To verify the ecological patterns that were inferred from this analysis, we used the empirically obtained data set of the fish assemblages of the River Doubs provided by Verneaux (1973, 1981). The succession of biocoenoses in the River Doubs proves that the classic longitudinal zonation concepts cannot always be applied unequivocally to any river, but it does confirm that a zonational pattern, consisting of biocoenoses, occurs in near-natural rivers. The analysis of the ecological patterns in the Doubs data set corroborates the patterns found in the Rhine-Meuse data set. Both analyses also confirm and elaborate the patterns in ecological guild structures that can be derived from the older zonation concepts and some of the predictions of the River Continuum Concept.

The species richness per zone increases downstream, from the Trout zone to the Barbel (Doubs) and Bream (Rhine-Meuse) zone, and decreases further downstream. The number of ecological guilds also increases downstream, and there are clear shifts in the structure of the guilds. This general pattern is found in many large rivers (Huet, 1949; Horwitz, 1978; Holčík, 1989; Morin & Naiman, 1990; Peňáz & Jura-

Table 3. Fish community of the river Doubs (France). Presence in biocoenoses and fish zones (1 = species present, 1* = typological preferendum) (Verneaux, 1973, 1981); resistance index (Ir), pollution index (Ip) (Verneaux, 1973, 1981), and indices of overall eurytopy in current waters (Ec) and standing waters (El) and index of reproductive habitat eurytopy in current or standing waters (Er, max) (Grandmottet, 1983)

Species	Fish zones														
	Spring			Trout			Grayling	Barbel	Bream	'Smelt'					
	Biocoenoses										Sensitivity indices				
	B0	B1	B2	B3	B4	B5	B6	B7	B8	B9	Ir	Ip	Ec	El	Ermax
Salvellinus fontinalis		1	1*	1	1						3		0.3		0.2
Cottus gobio		1	1	1*	1	1	1				3		0.13		0.11
Salmo trutta fario		1	1	1	1*	1	1	1			5.5	6	0.29		0.14
Phoxinus phoxinus			1	1	1*	1	1	1			4.5		0.32		0.33
Thymallus thymallus				1	1	1*	1	1			3	3	0.34		0.11
Barbatula barbatulus			1	1	1	1*	1	1	1		7	7	0.19		0.57
Zingel asper					1	1	1*	1			3.5				
Chondrostoma nasus						1	1*	1	1		6		0.21		0.21
Chondrostoma toxostoma						1	1*	1	1		6		0.22		0.21
Leuciscus souffia agassizi					1	1	1*	1			4		0.37		0.26
Lota lota						1	1	1*	1		4		0.2		0.08
Leuciscus leuciscus						1	1	1*	1		4.5	5	0.39		0.26
Alburnoides bipunctatus						1	1	1*	1		5		0.41		0.26
Gobio gobio					1	1	1	1*	1	1	5.5		0.41		0.26
Barbus barbus						1	1	1*	1		5	4	0.32		0.26
Leuciscus cephalus					1	1	1	1*	1	1	7	7	0.62		0.71
Esox lucius							1	1	1*	1	5.5			0.19	0.06
Rhodeus sericeus amarus							1	1	1*	1	5.5	5		0.28	0.11
Cyprinus carpio								1	1*	1	6			0.35	0.19
Perca fluviatilis							1	1	1*	1	5	4		0.46	0.29
Lepomis gibbosus							1	1	1*	1	5.5	5		0.52	0.37
Gymnocephalus cernuus								1	1*	1	7			0.71	0.45
Stizostedion lucioperca								1	1*	1	7			0.46	0.45
Alburnus alburnus								1	1*	1	7.5	6.7		0.85	0.71
Rutilus rutilus							1	1	1*	1	8	6.7		0.87	0.77
Ictalurus melas									1	1*	6.5	5.6		0.17	0.04
Scardinius erythrophthalmus									1	1*	6	6		0.22	0.14
Tinca tinca							1	1	1	1*	6.5			0.38	0.19
Micropterus salmoides									1	1*	4.5			0.29	0.4
Blicca bjoerkna								1	1	1*	7.5	6.7		0.55	0.46
Abramis brama								1	1	1*	7			0.52	0.66

Table 4. The mean Resistance Index (Ir) of the species assemblages inhabiting the Biocoenoses and Fish Zones of the River Doubs, calculated from data in Verneaux (1973, 1981)

Biocoenosis	B1	B2	B3	B4	B5	B6	B7	B8	B9
Mean Ir	3.83	4.60	4.30	4.60	4.90	5.21	5.71	6.04	6.32
Fish Zone	SPRING			TROUT		GRAYLING	BARBEL	BREAM	"SMELT"
Mean Ir	4.22			4.60		5.21	5.71	6.04	6.32

jda, 1993). The River Continuum Concept (Vannote et al., 1980) predicted maximum biotic diversity in midsize streams (river orders 4–6) in response to maximum habitat diversity and environmental variation (Morin & Naiman, 1990). Our data seem to confirm this prediction, in that maximum taxonomic richness (expressed as the number of taxonomic orders or as the number of species) and maximum number of feeding guilds are encountered in the Barbel and Bream zones.

According to the River Continuum Concept autochthonous production increases and allochthonous sources of carbon decrease downstream in large rivers, which should result in an increase in zooplanktivorous and phytivorous species and a decrease in benthivorous species (Bayley & Li, 1992). Our results comply with this rule (Fig. 4), with the possible exception of the Smelt zone (which is characterized by low abundances of aquatic macrophytes). According to Horwitz (1978) the proportion of detritivores should increase downstream. The Doubs data set is in accordance with this postulate, but the proportion of detritivores in the Rhine-Meuse data set does not show a clear trend; however, the number of detritivorous species per guild clearly increases downstream in the Rhine-Meuse. It is interesting to note that an extensive study by Morin & Naiman (1990) failed to corroborate Horwitz's postulate.

Guilds

The concept of guilds is already well-established in scientific fish studies, but could be applied on a much wider scale in everyday nature management, especially because important inferences about habitat quality can be drawn from its use.

Grouping fish species into ecological guilds can be a useful method for assessing the ecological integrity and functioning of large river systems. Shifts in the structure of functional groups as a result of environmental degradation can be explained by general theories of river ecology, geomorphology and chemistry, that can also set guidelines for ecological restoration of degraded river systems, by elucidating the natural configuration of riverine habitats and processes. Balon's classification of fish species according to reproductive strategy is now applied worldwide; it would be interesting to test the applicability of the flow preference classification devised by Schiemer & Waidbacher (1992) for European rivers and elaborated by Schouten & Quak (1994) for the Dutch situation in river systems in other continents. There is plenty of scope for more detailed elaborations of these basic classifications (e.g. the refined classification of reproductive strategies of Vriese et al. (1994)), which will enhance their usefulness, especially in local and regional studies. Feeding guilds of fishes can be used to test the predictions of the River Continuum Concept (Vannote et al., 1980), although it will be difficult to find undisturbed reference rivers that still constitute natural continua, as at present 70% of the rivers in the northern third of the world have anthropogenically altered flow regimes and are more or less fragmentated (Dynesius & Nilsson, 1994). The European fish fauna mainly consists of generalist feeders (Oberdorff & Hughes, 1992); therefore our analyses of fish zones based on feeding guilds were not very discriminating.

Because guilds are human constructs, and not real entities, assigning species to guilds can sometimes be problematic. For instance, the diet of a fish species changes according to time of the year, time of the day, water levels, habitat, length and age (Lelek & Köhler, 1989). In general, diet varies according to prey availability and biotic interactions in the fish assemblage the species is part of (Bergers, 1991). Generalist feeders are able to switch their diet according to the prey availability in the habitat they are in, whereas specialist feeders are more reliant on the availability of specific habitats. Generalist feeders experience a high degree of interspecific competition, but a low degree of intraspecific competition, whereas the competition situation is the reverse in specialist feeders (Bergers, 1991).

The species in different guilds are also characterized by different levels of intrinsic capacity to withstand environmental degradation, expressed by the resistance index Ir of Verneaux (1973, 1981), and different levels of habitat-flexibility, expressed by the index of euryoecy E of Grandmottet (1983). Rheophilic and limnophilic fish are generally sensitive to perturbations of their environment (low Ir) and can be characterized as habitat specialists (low E). Eurytopic fish species are more tolerant with regard to environmental degradation (high Ir) and can reproduce in many habitats (high E).

The use of fish data

Two fairly recent techniques for analyzing fish fauna that apply ecological guild classifications could not be used in this study because they have not yet been used extensively in the Netherlands, and hence not enough data were available. The first one is the Index of Biotic Integrity (IBI; Karr, 1981; Simon, 1999). In the IBI-methodology the species richness, guild structure and health of fishes in the study area are compared with those of the same area in a pre-disturbance reference situation. An important feature of the IBI-methodology is that fish species are labelled as 'sensitive' or 'tolerant'. The resistance index of Verneaux (1973, 1981) quantifies this sensitivity, and incorporation of this index in the IBI-methodology could lead to its improvement. The same can be said of Grandmottet's (1983) index of euryoecy.

The second assessment technique is the sampling of solely young-of-the-year fishes (YOY or 0+), to evaluate the suitability of river and floodplain areas as spawning and nursery habitats (Copp, 1989; Schiemer & Spindler, 1989; Copp et al., 1991). The egg, larval and juvenile phases are the most critical stages in the life history of fishes, so YOY sampling is a more sensitive indicator of habitat suitability than the sampling of adult fishes (Copp et al., 1991; Quak, 1994). The IBI uses data on adult fishes, but some authors (Copp et al., 1991; Berrebi dit Thomas et al., 1998) have suggested that data on YOY fishes could be incorporated to enhance use of the IBI in the relatively undifferentiated, species poor European waters. However, inclusion of YOY data tends to inflate the IBI-scores, and this is why in America only data on adult fish are included in the IBI (Simon, 1999). Also, the IBI methodology is based on comparison of the present state of a water body with its reference state; information on the YOY structure of that reference state may be scarce or non-existent. Moreover, YOY assemblages give no information about the importance of a water body for fish species that do not spawn in the area itself but for which it may be an important feeding or wintering habitat, such as diadromous species.

Red Lists of threatened and vulnerable species

The combined impact of chemical, physical and biological disturbances brought about by man in most river systems has affected the various fish species unevenly: guilds of specialized species that share life history strategies that are highly adapted to specific riverine conditions have declined far more than generalist species that can survive in a wide range of habitats that are not characteristic of natural river ecosystems (Figs 5, 6 and Table 2). Our analyses thus corroborate the postulate of Hengeveld (1996) that species do not become extinct randomly, but that species under threat often have certain life history traits in common. For instance, 78% of the rheophilic species are on the Red List. The migratory rheophils were often the first to become extinct, their decline was already well underway in the 19th century (De Nie, 1996, 1997; De Groot, 2002). Non-migratory river fishes (rheophilic A2 and B) have become (very) rare because their lotic habitats are gone or degraded (Grift et al., 2000; Grift, 2001). Limnophilic river fishes, that are dependent on clear waters with aquatic macrophytes, have become rare, mainly as a result of eutrophication. The status of many limnophilic species is worse in the rivers than in other aquatic habitats, and their national Red List status as 'not threatened' in the Netherlands is not indicative of the present decline of these phytophilic spawners in riverine habitats (De Nie, 1997). The same situation applies to rheophilic C species (e.g. Smelt, Flounder): closing of the river mouths by dams and sluices has made these species rare in the rivers, but because they are still abundant along the sea shore and in Lake IJsselmeer, they are not threatened on a national level. An example of the poor conditions for reproduction in regulated large rivers is presented by Vriese et al. (1994), who assessed the present availability of spawning and nursery areas in the Dutch part of the River Meuse for 21 fish species. Their study revealed that obligatory phytophilic spawners suffer a severe lack of habitat for reproduction, because in only 1.5% of the river stretches examined aquatic macrophytes were present. Rheophilic species completely lack reproduction habitats (functional gravel banks with a relatively high rate of flow) due to channelization and dam construction. Only species that can reproduce under a wide range of the environmental conditions find sufficient reproduction habitat in the completely altered river. The fish fauna is presently dominated by eurytopic polyphils and phytolithophils (Admiraal et al., 1993; Raat, 2001); nearly all eurytopic species are unthreatened, though eutrophication is disadvantageous for the phytophilic spawners within this group. Piscivores occur at unnaturally high levels (Lelek, 1991; Lelek & Buhse, 1992).

In a natural river system each zone has a different constellation of specific habitats, characterized by a unique assemblage of fish species that form a successional series in the longitudinal direction of the river. Because of the decline of the sensitive, stenoecious species in all four rheophilic guilds and in the limnophilic guild, this differentiated longitudinal zonation is hardly recognizable anymore in heavily impacted large rivers such as the River Rhine.

Because of the differentiated nature of the fish zones and the unique biogeographic history of every river system, it would be desirable to have Red Lists of threatened and vulnerable fish species (and subspecies) on the basis of whole catchments of rivers, rather than Red Lists determined by national boundaries, that cut up transboundary river systems (Kirchhofer & Hefti, 1996). To enhance the applicability of Red Lists as tools for conservation and restoration, regional Red Lists could be drawn up for the various fish zones within these catchments.

Fish zonations and guilds as the basis for the assessment of the ecological integrity of large rivers

Human disturbances can cause shifts in the zonation pattern and biocoenotic characteristics of a river ('rhithralisation and potamalisation effects', Jungwirth et al., 1995), and anthropogenic pressure in general can cause shifts in the species composition within a guild, or even the complete disappearance of a guild. Within a specific fish region, a suitable way of analyzing the impact of human disturbance on the structure of the fish community is by applying guild classifications and comparing the guild structure of the present state of a fish zone with that of the reference situation (Schmutz et al., 2000). This method of assessing the ecological integrity of running waters can be applied to detect medium- and high-dose human alterations to river systems on regional, national and catchment scales, for instance for the large-scale monitoring programmes specified for the European Water Framework Directive (EC, 2000). The general method can be adapted to all river types, but just like the IBI methodology, it requires adaptations that reflect the specific biogeographic character of the river under study (affecting the classifications of biocoenoses and guild structures), and it requires sufficient knowledge of the local reference situation. Assessment systems based on the use of biocoenotic regions and guild classifications are being implemented for macrofauna and fish in Germany (Schöll & Haybach, 2000) and Austria (Chovanec et al., 2000). The fish guild classifications used in this study could contribute to the multi-level concept for fish-based assessment of the ecological integrity of rivers (Schmutz et al., 2000; Verneaux et al., 2003).

Potentials for nature management and conservation

On a European level, the protection of the fish in the upper Trout zone (epirithral) does not seem to be too dificult. There are certainly enough small water courses with a natural hydrological and morphological structure of the stream bed and its surroundings available in the sparsely populated mountain areas of Europe (Lelek, 1987). A more complicated and urgent problem seems to be the protection and conservation of the species inhabiting the lower Trout (metarithral), Grayling and Barbel zones (Lelek, 1987; Schiemer, 1988). These stretches are inhabited by sensitive, stenoecious rheophilic fish species and these areas are nearly everywhere substantially changed or mostly destroyed with respect to their physical and biological characteristics, particularly by the building of weirs for the supply of energy and water for small industries. Now that those ancient weirs are no longer needed, their continued maintenance should be reconsidered. The former natural habitats can never be fully restored, but the remaining habitats still contain a very diverse and species-rich fish community, which is very valuable for conservation.

Although the Bream zone is inhabited by fish species that are relatively tolerant (high Ir and Ip) and euryoecious (high E), conservation and restoration measures in this zone are even harder to pursue than in the upstream zones, because in many large rivers in Europe, this zone is more heavily impacted by pollution and regulation (Copp et al., 1991; Peňáz & Jurajda, 1993). There is no hope of recovering the natural situation, because of the enormous economic and safety interests in this densely populated region (Nienhuis & Leuven, 2001). The main river has been channelized and normalized to make it navigable and to provide a rapid, unobstructed discharge of water and ice. Aquatic and riparian vegetation are almost entirely absent. The floodplains have been reduced and immobilized by summer and winter dikes for agricultural purposes, and they are now covered by thick layers of deposited clay (Lenders et al., 1999). The natural transversal gradient in inundation frequency has ceased to exist, the floodplains are more or less isolated from the main river channel (Van den Brink, 1994). Recently, agricultural practises in the floodplains of Dutch large rivers have become relatively unprofitable, allowing rehabilitation of the floodplains to a more natural state, e.g. through removal of summer dikes and clay layers (Leuven et al., 2002; Nienhuis et al., 2002). Natural habitats are being re-created, and especially active secondary channels (which had become entirely absent in the Dutch river area) are thought to be very important for typically riverine fish species, because they provide a lotic component, that could replace the lost lotic habitat of the main river channel (Grift et al., 2000; Grift, 2001; Buijse et al., 2002).

Conclusions

The informational value of fish catch data can be enhanced by application of ecological fish guild classifications and indices for sensitivity and habitat flexibility.

Including information on ecological fish guilds can enhance the usefulness of fish zonation concepts, in a way that they can be used as tools for assessment and management of the ecological integrity of large rivers.

There is plenty of scope for further refinement of existing fish guild classifications and the development of new classifications based on other life-history traits. It is important for the conceptual advancement that these classifications are published in international journals.

Flow preference and reproduction ecology of river fish are closely linked.

Because the European fish fauna mainly consists of feeding generalists, the discriminative abilities of simplistic feeding guild classifications are not very high. More elaborate feeding guild classifications might be able to improve on this.

In rivers, the fish species richness per zone increases downstream, from the Trout zone to the Barbel or Bream zone, and decreases further downstream. The number of ecological guilds also increases downstream, and there are clear shifts in the structure of the guilds. The proportion of rheophilic species in the fish community decreases downstream, and the proportions of limnophilic and eurytopic species increase. Lithophilic and psammophilic spawners are dominant in the upper zones, whereas the lower zones are dominated by phytophilic and phytolithophilic spawners. The Smelt zone, which includes the estuaries, is dominated by rheophilic species that are lithophilic or pelagophilic spawners. The proportion of zoobenthivorous and periphytivorous species decreases downstream, and the proportion of zooplanktivorous and phytivorous species increases.

Most Red List species occur in the Trout zone, the Bream zone has the least Red List species.

Red Lists of threatened freshwater fish species should preferably be drawn up for each individual large river catchment, not (or: not only) for each individual nation as a whole.

The combined impact of chemical, physical and biological disturbances brought about by man in most river systems has affected the various fish species unevenly: guilds of specialized species that share life history strategies that are highly adapted to specific riverine conditions have declined far more than generalist species that can survive in a wide range of habitats that are not characteristic of natural river ecosystems.

Because of the decline of the sensitive, stenoecious species in all four rheophilic guilds and in the limnophilic guild, and the subsequent over-abundance of the generalist species of the eurytopic guild, the original longitudinal fish zonations are hardly recognizable anymore in heavily impacted large rivers such as the River Rhine. Hence these rivers do not meet the criteria for ecological integrity.

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Characterisation of high-altitude *Artemia* populations from the Qinghai-Tibet Plateau, PR China

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Abstract

The brine shrimp *Artemia* was discovered in a number of saline lakes on the Qinghai-Tibet Plateau, widely diverging in chemical composition. Several lakes were athalassohaline, with relatively high amounts of trace elements. Common environmental factors are their high altitude (exceeding 4500 m) and the low average annual temperatures. A number of *Artemia* populations in this area were analysed to assess their preference for low temperatures and an athalassohaline medium. Furthermore, their characteristics were compared with *Artemia tibetiana*, the species recently described for one lake in this area. All samples contained a variable mixture of parthenogenetic and bisexual individuals. A cross-breeding test of the sample from Jingyu Lake showed cross-fertility both with *A. tibetiana* and *A. sinica.* All populations showed similarities to *A. tibetiana*: a large cyst diameter and naupliar length, high HUFA content and a high tolerance to low temperatures, as compared to the control *A. franciscana* samples. These can thus be considered as recurrent characteristics of the populations from the high-altitude low-temperature environment on the Qinghai-Tibet Plateau, although further research is needed to identify their exact species status.

Introduction

The Qinghai-Tibet Plateau, located in southwest China, has an average elevation exceeding 4500 m. About 350 saline lakes are situated in this area and have a wide diversity of geological background and chemical composition (Zheng et al., 1993).

About 16 *Artemia* sites on the Qinghai-Tibet Plateau are reported by Xin et al. (1994) and Zheng (1997). New habitats are being explored and new cyst material is being collected. However, knowledge about these particular *Artemia* biotopes advances at a rather slow pace, due to the difficult accessibility of the area and the logistic problems for cyst harvesters. Consequently only limited amounts of samples have been collected and analysed. Unfortunately their exact origin and background are not always easy to trace, which may hinder the interpretation of analytical results. Finally, inconsistent transcription and random use of local Tibetan and/or Chinese toponyms add to the confusion.

As compared with the lakes elsewhere in China, these saline lakes show higher contents of trace elements such as B, Li, Cs, Rb and As (Zheng, 1997), to the extent that commercial extraction of lithium and boron is a local industrial activity (e.g. in Lake Zabuye) (Zheng, 2002). This environment is further characterized by its high altitude (as high as 4900 m), and low temperatures (average annual air temperature between –5 and +1 °C; Zheng, 1997).

Insofar as Chinese scientific research is accessible to the international community, most studies focused on the *Artemia* population from Lagkor Co (Liu et al., 1998ab). Through a multidisciplinary approach

Abatzopoulos et al. (1998, 2002) identified this population as a new bisexual species, *Artemia tibetiana.* Sun et al. (1999), using Amplified Fragment Length Polymorphism (AFLP) of different Chinese *Artemia* species and strains, succeeded in differentiating *A. tibetiana* from *A. sinica*, the other bisexual species from continental China. Han et al. (1999) illustrated the different fatty acid metabolism of this species after enrichment, in comparison with *A. franciscana*. Clegg et al. (2001) reported on its high sensitivity for heat stress, probably an adaptation to its cold natural environment.

Field data of the brine shrimp population of Lagkor Co or any other population on the Plateau are extremely scarce and are generally limited to momentary observations (Zheng, 1997). The species status of the populations in other lakes has also not been confirmed. It is not clear to what extent the characteristics, as previously described for *A. tibetiana*, also occur in other *Artemia* populations from the same area. This study aims to contribute to the knowledge of the anostracan biodiversity in this area, by analysing the characteristics of a number of *Artemia* samples from the Qinghai-Tibet Plateau that have recently become available.

Materials and methods

Cyst samples

Seven samples originating from the Qinghai-Tibet plateau were studied (Table 1). The number of analyses run on each sample depended on the available cyst quantity and their hatching percentage. The exact geographical background of one sample ('Tibet A') was unknown.

Biometrical characteristics

The cyst diameter, chorion thickness and nauplius length were determined (n = 100), according to the methodology as described in Vanhaecke & Sorgeloos (1980).

Nutritional content: level of highly unsaturated fatty acids (HUFA's)

The fatty acid composition of the *Artemia* nauplii was analysed by a direct transmethylation method according to a modified procedure of Lepage & Roy (1984). The resulting fatty acid methyl esters (FAME) were separated and identified on a Chrompack CP 9001 gas chromatograph equipped with autosampler and a temperature programmable on-column injector (TPOCI). Identification was based on standard reference mixtures (Nu-Chek-Prep, U.S.A.); integration and calculations were done with the Maestro, Chrompack, software program.

Impact of environmental conditions

A number of laboratory tests were performed to assess the adaptation of the strains to the conditions as prevailing in their natural habitat: temperature, salinity and ionic composition of the medium.

a) hatching at different temperatures

The samples of Tibet A, Bong Co, Jingyu Lake, Haiyan Lake and Co Qen were hatched in triplicate at standard hatching conditions (35 g.l^{-1}, Instant Ocean® synthetic sea salt mixture, continuous illumination of 2000 lux provided by TL lamps; Lavens & Sorgeloos, 1996), but at different temperatures: 10, 16, 21 and 28 ± 0.5 °C. These temperatures were obtained by installing the 800 ml glass cylindroconical hatching recipients in an air-conditioned room (10 and 16 °C) or in a heated water bath (21 and 28 °C). The hatching process was followed over a total incubation period of 144 h, with measurements taken every 24 h. *Artemia franciscana* cysts (San Francisco Bay, SFB, California, USA; ARC code 1258) were used as control.

b) hatching in medium of different salinity, ionic composition and temperature

Tibet A and Lagkor Co cysts were hatched in the thalassohaline Dietrich & Kalle artificial seawater (Parsons et al., 1984) and in an artificially made Lagkor Co water (Table 2) using a simplified formula, based on ionic data as provided by Zheng (1997) (detailed information about the ionic composition of the other lakes was not available). The hatching was assessed at salinities of 15, 35 and 80 g.l^{-1} for each medium. This comparative test was run both at 21 and 28 ± 0.5 °C (by use of a heated water bath). All other conditions were standard (Lavens & Sorgeloos, 1996) and identical to the previous test. The test was run in triplicate for each combination of variables. Hatching was followed over a total incubation period of 48 h, with measurements taken every 24 h. *A. franciscana* cysts (Great Salt Lake, GSL, Utah, U.S.A.; commercial batch) were used as control, as the San Francisco

Table 1. Cyst samples: origin, ARC cyst code, geographical and hydrochemical parameters (Zheng, 1997, and data provided by Salt Research Institute, Tanggu, China); H% = hatching percentage of raw sample in standard conditions (Lavens & Sorgeloos, 1996) upon arrival at ARC

	ARC code	Elevation (m)	Surface area (km^2)	Longitude (E)	Latitude (N)	Ionic composition	H%
Tibet A (unknown origin)	1346	–	–	–	–	–	62.4
Lagkor Co	1348	4490	92	84 °13′	32° 03′	Carbonate	11.8
Bong Co	1462	4664	140	91° 09′	31° 13′	–	6.7
Bozi Co	1461	4663	25	86° 07′	30° 28′	–	21.7
Jingyu Lake	1524	4720	300	89° 09′	36° 03′	$MgSO_4$	65.6
Haiyan Lake	1525	–	–	100° 11′	36° 03′	–	54.8
Co Qen	1526	–	–	85° 09′	30° 59′	Na_2SO_4	36.8

Bay sample (ARC code 1258) had shown low hatching in the previous test.

c) survival in medium of different salinity, ionic composition and temperature

Tibet A cysts were hatched at standard conditions (35 $g.l^{-1}$ Instant Ocean® artificial seawater, 28 °C; Lavens & Sorgeloos, 1996). Two hundred instar I nauplii were transferred into glass cylindroconical recipients with 800 ml culture medium, and grown for a total period of 17 days at the same combinations of temperature, salinity and ionic composition as described under b (the lowest salinity, 15 $g.l^{-1}$, was not included in this test). As the instar I nauplius is resistant to osmotic shocks (Sorgeloos, 1980), no acclimation period was provided. Bottom aeration was provided continuously and a 12/12 photoperiod (light intensity 2000 lux) was maintained. Each treatment was run in triplicate. The test animals were fed a standard diet, based on the unicellular alga *Dunaliella tertiolecta* Butch (adapted to the respective salinities) and the yeast-based Lanzy PZ® (INVE N.V., Belgium) (Coutteau et al., 1992; Nguyen Thi, 2000). Survival was determined (and water renewed) at day 4, 8, 11, 14 and 17.

d) heat shock test

Cysts from Jingyu Lake and Co Qen were subjected to a heat shock test, as described by Clegg et al. (2001). Hydrated cysts were gradually heated from 22.0 to 50.0 °C. After being maintained for 15, 30, 60 and 80 min at 50.0 °C, the cysts were incubated again at 22.0 °C and the hatching was assessed. Cysts from Lagkor Co, San Francisco Bay (ARC code 1364) and Vietnam (Vinh Chau, ARC code 1349) were included as reference strains, in order to compare with available literature data.

Table 2. Formulation of Lagkor Co and Dietrich & Kalle artificial media; salt contents of stock solution ($g.l^{-1}$ medium; to be diluted to experimental salinities)

Salt	Lagkor Co	Dietrich & Kalle
NaCl	11.64	67.90
$MgCl_2 \bullet 6H_2O$	11.34	30.77
$CaCl_2$	0.43	3.27
KCl	8.56	1.94
$Na_2SO_4 \bullet 10\,H_2O$	111.40	25.74
$Na\,HCO_3$	6.84	1.14
H_3BO_3	4.19	0.008
Na_2CO_3	3.66	–

Species characterization of strains

a) sex ratio

In order to assess the type of reproduction (parthenogenetic or bisexual), cysts of all populations (except Lagkor Co, which had been defined as *A. tibetiana*) were hatched in standard conditions (Lavens & Sorgeloos, 1996). Instar I nauplii of each sample were subsequently cultured in 800 ml glass cylindroconical recipients. The test was performed at 21 ± 1 °C in 80 $g.l^{-1}$ Instant Ocean® artificial seawater, 2000 lux illumination with photoperiod 12/12, with a diet of exclusively *Dunaliella tertiolecta* (Coutteau et al., 1992). From the age of seven days onwards, 100–120 animals of each sample were raised individually in 50 ml Falcon tubes for a period of 1 month (or until all animals had died) in the same culture conditions. Water was renewed twice a week. When sexual maturity was attained, the number of males and females was determined, as well as the number of females releasing nauplii and/or cysts and ovigerous females that didn't spawn. In case of cyst release, the viability of the offspring was assessed by hatching the cysts in standard conditions (Lavens & Sorgeloos, 1996) after two weeks storage in brine at –18 °C to break diapause.

b) cross-breeding

In view of the mixed status of the samples (as revealed by the sex ratio test), a cross breeding test was only performed with the Jingyu Lake sample, which had the lowest fraction of parthenogenetic females. Reciprocal crosses of the Jingyu strain were performed with *A. tibetiana* (Lagkor Co, ARC code 1348), *A. sinica* (Yuncheng, China, ARC code 1218) and *A. franciscana* (San Francisco Bay, USA, ARC code 1364) (see Table 7 for design of crosses).

Cysts were hatched in standard conditions, and nauplii were individually raised in 50 ml Falcon tubes (21 ± 1 °C, 80 $g.l^{-1}$ Instant Ocean® water, 2000 lux illumination, photoperiod 12/12, standard diet of *Dunaliella tertiolecta*). As soon as sexual differentiation occurred, males and females were paired (n = 10) and couples were raised separately in 50 ml Falcon tubes in the same culture conditions. Twice per week water was renewed and F1 offspring (cysts or nauplii) was counted. F1 cysts were stored at –18 °C for a minimum period of 2 weeks, while dehydrated in 300 $g.l^{-1}$ brine, to break the state of diapause. After an acclimation period of one week at room temperature, F1 cysts were hatched in standard conditions, and if sufficient animals were available, animals were paired (n=10) following the same procedure as for the parental generation. Ovoviviparously generated F1 nauplii were directly raised in the same conditions, and used for further crossing, if available in sufficient numbers. As the offspring of each set of 10 replicates was pooled, cross-fertility was assessed by the hatching percentage of the cysts produced, the encystment rate, and the number of ovoviviparously reproducing females in each combination.

Reciprocal crosses were continued until the F3 generation for those combinations where sufficient animals were produced. Each generation was cultured, and the offspring (cysts or nauplii) counted, for a maximum period of 30 days (or until death of all individuals). Dead individuals were replaced from the stock of individually cultured animals for the first two weeks; if males died after this period, the surviving female was further monitored.

Table 3. Biometric data of cyst samples (n=100). Values within the same column, sharing the same superscript, are not significantly different (one-way ANOVA at p = 0.05)

Sample	Cyst diameter (μm)	Chorion thickness (μm)	Instar I naupliar length (μm)
Tibet A	306.3 ± 20.3^{bc}	5.7	590.5 ± 36.1^{b}
Bong Co	295.7 ± 14.8^{d}	3.6	Not analysed
Bozi Co	284.5 ± 16.4^{e}	3.8	Not analysed
Jingyu Lake	320.0 ± 13.7^{ab}	13.3	607.1 ± 34.6^{a}
Haiyan Lake	291.2 ± 14.3^{d}	13.3	540.2 ± 31.6^{d}
Co Qen	312.1 ± 19.6^{abc}	11.2	558.3 ± 35.5^{c}

Statistical processing

Differences in cyst diameter and instar I naupliar length between strains were analysed by one-way ANOVA.

For the Great Salt Lake, Lagkor Co and Tibet A samples, the effects of ionic composition, temperature and salinity on hatching after 24 h hatching incubation were tested by a three-way ANOVA. The same analysis was performed with the 48 h hatching incubation data.

For the Tibet A sample, the effects of ionic composition, temperature and salinity on survival after 4, 8, 11, 14 and 17 days of culture were tested by three-way ANOVA's.

All data were tested for normality and homogeneity of variance before ANOVA was done. Tukey's Honest Significant Difference (HSD) test was performed to identify differences among means and significance was accepted at p<0.05.

Results

Biometrical characteristics (Table 3)

Average cyst diameters ranged between 284.5 μm (Bozi Co) and 320.0 μm (Jingyu Lake). Significant differences (one-way ANOVA, p < 0.05) in cyst diameter were found between the samples, with the

Table 4. HUFA analysis of cyst samples

FAME (mg.g^{-1} dry weight)	Lagkor Co	Bong Co	Bozi Co	Jingyu L.	Haiyan L.	Co Qen
18:2(ω-6)t	0.8	0.6	0.6	0.5	0.5	0.5
18:2(ω-6)c	5.4	5.7	4.5	5.9	5.7	6.6
18:3(ω-3)	7.5	0.2	0.5	4.9	12.1	4.4
20:4(ω-6)	7.4	2.1	3.1	4.4	1.8	2.5
20:5(ω-3)	43.0	29.4	21.6	42.7	31.7	30.7
22:6(ω-3)	0.7	0.5	0.6	0.9	0.3	1.1
$\Sigma(\omega\text{-}3) \geq 20{:}3(\omega\text{-}3)$	45.0	31.2	23.2	45.0	33.8	33.1
$\Sigma(\omega\text{-}6) \geq 18{:}2(\omega\text{-}6)$t	15.5	8.8	9.0	11.2	8.3	9.9

Jingyu Lake, Co Qen and Tibet A samples being significantly bigger, and the Bozi Co sample significantly smaller than the others.

Average instar I naupliar length ranged between 540.2 μm (Haiyan Lake) and 607.1 μm (Jingyu Lake). All values (4 samples analysed) were significantly different from one another (one-way ANOVA, $p < 0.05$). Both low (3.6 μm in Bozi Co) and high (13.3 μm for Jingyu and Haiyan Lakes) values were recorded for the chorion thickness.

Nutritional content: level of highly unsaturated fatty acids (HUFA's) (Table 4)

Cyst samples showed total ω-3 HUFA levels ($\geq$20:3(ω-3)) ranging between 23.2 (Bozi Co) and 45.0 mg.g^{-1} dry weight (Lagkor Co and Jingyu Lake). Levels of 20:5(ω-3) (eicosapentaenoic acid, EPA) ranged between 21.6 and 43.0 mg.g^{-1} dry weight for Bozi Co and Lagkor Co cysts, respectively. The Co Qen sample showed the highest value of 22:6(ω-3) (docosahexaenoic acid, DHA): 1.1 mg.g^{-1}. The values for other HUFA's with aquaculture relevance, e.g. linoleic acid 18:2(ω-6), linolenic acid 18:3(ω-3) and arachidonic acid 20:4(ω-6), were variable.

Impact of environmental conditions

a) hatching at different temperatures (Fig. 1)

Samples showed very different hatching levels (see Table 1) under standard conditions. To facilitate comparison values for each sample were plotted as a fraction (%) of the reference value obtained at the standard temperature 28 °C after 48 hr. At 10 °C, Tibet A, Jingyu, Haiyan and Co Qen had started hatching after 72 h incubation period. The first hatching for Bong Co at this temperature was observed at 96 h, whereas the first hatching for SFB only occurred at 120 h. Except for Co Qen and SFB, the final hatching obtained in the range 10–21 °C was higher or similar than at the reference temperature of 28 °C. Generally, for all samples analysed, the hatching rate was delayed at lower temperatures.

b) hatching in medium of different salinity, ionic composition and temperature (Table 5)

After 24 h hatching incubation, both temperature and salinity had a significant effect on hatching (three-way ANOVA, $p < 0.05$) of all samples, whereas the type of medium only had a significant effect for the GSL strain. For all strains there was a significant interaction after 24 h incubation between hatching salinity on one hand, and ionic composition and temperature on the other. There was no significant interaction between ionic composition and salinity.

After 48 h hatching incubation, there was a significant effect ($p < 0.05$) of all factors and all interactions for all samples, with the exception of a non-significant temperature effect ($p > 0.05$) for GSL, and a non-significant interaction temperature vs. ionic composition for Tibet A.

The Great Salt Lake sample performed significantly better in the thalassohaline Dietrich & Kalle artificial medium, while both Tibet samples had higher hatching in the artificial Lagkor Co medium. In Dietrich & Kalle medium of 80 g.l^{-1}, both Tibet samples did not show any hatching at all within 48 h at both 21 and 28 °C. At lower salinities differences between both types of medium within each sample were non-significant ($p > 0.05$).

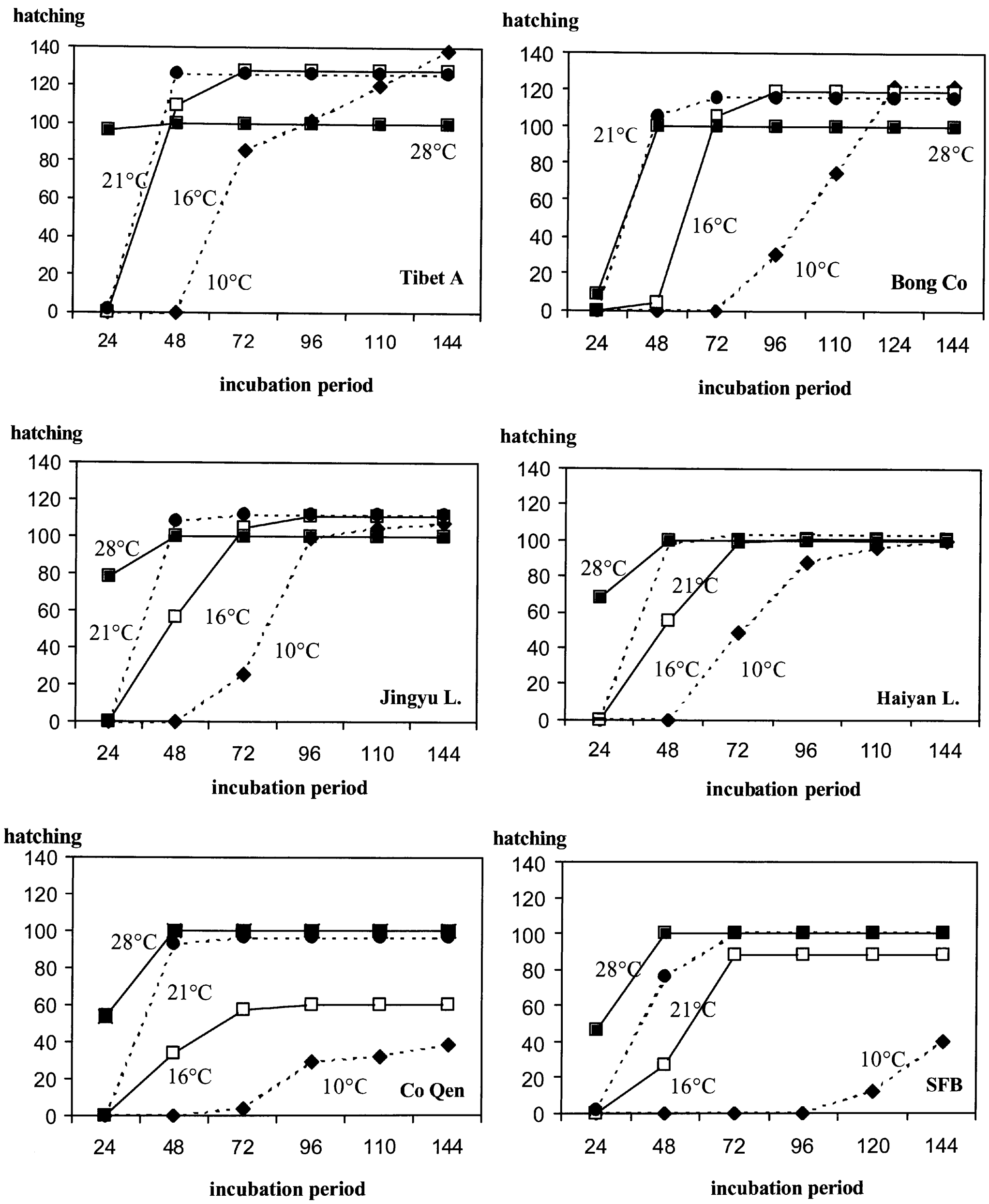

Figure 1. Hatching of cyst samples at different temperatures. Values are normalized to the value obtained at 28 °C after 48 hr (set as '100').

Table 5. Hatching in medium of different salinity, ionic composition and temperature. For each sample and incubation time (24 and 48 h) values with the same superscript are not significantly different (three-way ANOVA at $p = 0.05$); LC = Lagkor Co; DK = Dietrich & Kalle

Sample	Incubation time (hr)	Incubation temperature (°C)	$15\ g.l^{-1}$		$35\ g.l^{-1}$		$80\ g.l^{-1}$	
			LC water	DK water	LC water	DK water	LC water	DK water
Tibet A	24	21	42.3 ± 12.8^{cd}	51.1 ± 1.7^{bcd}	22.6 ± 2.8^{e}	19.1 ± 3.4^{e}	0^{f}	0^{f}
		28	73.9 ± 0.8^{ab}	71.8 ± 2.8^{ab}	72.3 ± 2.1^{ab}	59.5 ± 11.0^{abc}	0^{f}	0^{f}
	48	21	72.7 ± 2.1^{abc}	78.7 ± 2.0^{ab}	74.8 ± 1.4^{abc}	76.3 ± 1.3^{ab}	68.0 ± 2.0^{bc}	0^{e}
		28	78.6 ± 4.8^{ab}	72.7 ± 3.0^{abc}	77.3 ± 2.1^{ab}	68.2 ± 2.6^{bc}	36.2 ± 5.0^{d}	0^{e}
Lagkor Co	24	21	5.0 ± 3.3^{def}	15.7 ± 6.0^{bcd}	12.5 ± 8.0^{bcde}	0^{ef}	0^{ef}	0^{ef}
		28	24.7 ± 1.4^{ab}	21.0 ± 2.9^{abcd}	0^{ef}	0^{ef}	0^{ef}	0^{ef}
	48	21	63.9 ± 7.9^{bcd}	63.8 ± 6.4^{bcd}	66.5 ± 6.1^{abcd}	58.9 ± 4.2^{bcd}	0^{f}	0^{f}
		28	72.9 ± 2.8^{abcd}	82.9 ± 4.5^{ab}	70.9 ± 7.7^{abcd}	60.2 ± 11.5^{bcde}	36.1 ± 4.2^{de}	0^{f}
Great Salt Lake	24	21	85.4 ± 0.6^{a}	84.4 ± 2.3^{a}	87.2 ± 1.4^{a}	84.7 ± 0.2^{a}	17.5 ± 3.5^{c}	0^{d}
		28	86.1 ± 0.5^{a}	86.6 ± 1.7^{a}	88.6 ± 2.7^{a}	84.9 ± 2.6^{a}	27.2 ± 3.3^{b}	13.8 ± 6.7^{c}
	48	21	89.6 ± 1.2^{abcdef}	91.0 ± 1.0^{abcf}	89.8 ± 2.0^{abcdf}	89.5 ± 2.1^{abcdef}	24.9 ± 3.0^{i}	83.8 ± 3.1^{cdeg}
		28	89.0 ± 2.2^{abcdef}	87.5 ± 1.9^{abcdef}	84.6 ± 1.6^{bcdefg}	86.3 ± 1.9^{abcdefg}	34.6 ± 1.9^{h}	80.8 ± 1.7^{bcdeg}

Table 6. Survival (%) of Tibet A *Artemia* in medium of different salinity, ionic composition and temperature. For each culture period (days 4, 8, 11, 14 and 17) values with the same superscript are not significantly different (three-way ANOVA at $p = 0.05$); LC = Lagkor Co; DK = Dietrich & Kalle

Culture period		28 °C		21 °C	
		35 g.l^{-1}	80 g.l^{-1}	35 g.l^{-1}	80 g.l^{-1}
Day 4	LC water	66.3 ± 14.6^{abcd}	56.0 ± 20.1^{abcd}	77.2 ± 18.7^{abc}	69.0 ± 31.0^{abd}
	DK water	26.0 ± 7.0^{bcde}	3.0 ± 1.8^{cde}	50.5 ± 16.9^{abcde}	35.3 ± 9.8^{abcde}
Day 8	LC water	44.7 ± 15.3^{abde}	0^{cde}	62.2 ± 5.4^{abde}	48.7 ± 30.8^{abde}
	DK water	8.3 ± 3.9^{bcde}	0^{cde}	39.0 ± 17.4^{abcde}	19.8 ± 11.3^{bcde}
Day 11	LC water	16.7 ± 17.7^{bc}	0^{bc}	54.1 ± 6.9^{ab}	30.2 ± 22.2^{abc}
	DK water	5.8 ± 3.6^{bc}	0^{bc}	29.3 ± 15.3^{abc}	10.9 ± 9.0^{bc}
Day 14	LC water	0^{bc}	0^{bc}	42.9 ± 14.6^{ab}	22.7 ± 19.2^{abc}
	DK water	0^{bc}	0^{bc}	18.5 ± 12.6^{abc}	10.0 ± 0.85^{bc}
Day 17	LC water	0^{bc}	0^{bc}	39.0 ± 16.7^{ab}	16.3 ± 15.4^{ab}
	DK water	0^{bc}	0^{bc}	14.9 ± 9.7^{abc}	6.0 ± 0.42^{bc}

c) survival in medium of different salinity, ionic composition and temperature (Table 6)

There was a significant effect (three-way ANOVA, $p < 0.05$) of the ionic composition and temperature on survival of the Tibet A strain throughout the culture period. After 8 and 11 days of culture, there was also a significant effect of culture salinity on survival. Significant interactions between factors were only found for ionic composition vs. culture temperature at the end of the culture period (from 14 days onwards).

Generally, survival at the end of the test period was limited (39.0% was the highest final survival obtained). Survival was higher (though not always significantly) at 21 °C than at 28 °C: at the higher temperature, no live animals were observed after 2 weeks culture period in any medium, and in a thalassohaline medium of 80 g.l^{-1}, only 3% of the animals survived the first 4 days.

d) heat shock test (Fig. 2)

Viability of the cysts from Lagkor Co was minimal after the heat shock treatment, whereas the samples from Vietnam and San Francisco Bay proved most tolerant. The samples from Jingyu Lake and Co Qen took an intermediate position between those two extremes: about 50% of the cysts did not survive 15 min exposure to 50 °C, and a 60 min treatment resulted in complete inhibition of hatching for both strains.

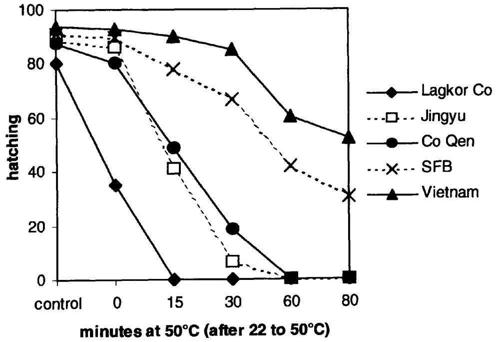

Figure 2. Hatching of cysts after heat shock of variable duration and subsequent incubation at 22.0 °C.

Species status of strains

a) sex ratio

The sex ratio (males/females) ranged between 45/55 and 40/60 for the Tibet A, Bozi Co, Bong Co and Jingyu Lake samples. For the samples from Haiyan Lake and Co Qen it was 32/68 and 11/89, respectively.

All samples had few ovoviviparously reproducing females. The status of the other females was unclear, as the large majority (>90%) had cysts in the uterus, but no spawning occurred. Only in Co Qen about 50% of the females with cysts in their uterus actually released cysts. The hatching percentage of this offspring after 2 weeks storage in brine at −18 °C was 23%.

Table 7. Cross breeding test of Jingyu (JY) strain with San Francisco Bay (SFB), Lagkor Co (LC) and Yuncheng (YC); n = 10; n.t. = not tested; H% = hatching percentage;% encyst = encystment rate; ovv. females = number of females (out of 10 replicates) producing partially or exclusively live nauplii. *F3 production by ovoviviparously generated F2 was not tested by lack of sufficient numbers of surviving F2 individuals

Cross (female × male)	F1 production (offspring/female/day)				
	cysts	nauplii	H% of cysts	% encyst	ovv. females
SFB × SFB	14.7 ± 8.8	8.6 ± 9.6	37.8	63.1	9
JY × JY	7.3 ± 6.8	1.8 ± 2.6	77.5	80.2	4
LC × LC	2.7 ± 2.8	2.1 ± 2.7	3.2	56.3	6
YC × YC	14.5 ± 8.5	9.8 ± 11.9	74.8	59.7	7
JY × SFB	2.7 ± 3.5	0	0	100	0
JY × LC	3.8 ± 4.0	0.5 ± 1.0	51.0	88.4	2
JY × YC	5.0 ± 4.1	3.2 ± 2.9	69.9	61.0	10
SFB × JY	14.6 ± 15.7	0	0	100	0
LC × JY	4.0 ± 3.6	1.0 ± 1.6	83.3	80.0	3
YC × JY	21.6 ± 11.3	1.9 ± 2.2	60.2	91.9	5

Cross (female × male)	F2 production (offspring/female/day) by oviparously generated F1					F2 production (offspring/female/day) by ovoviviparously generated F1				
	cysts	nauplii	H% of cysts	% encystm	ovv. females	cysts	nauplii	H% of cysts	% encystm	ovv. females
SFB × SFB	13.6 ± 7.1	3.4 ± 5.8	54.2	80.0	5	n.t.	n.t.	–	–	–
JY × JY	2.9 ± 2.5	1.1 ± 2.0	83.2	72.5	3	n.t.	n.t.	–	–	–
LC × LC	4.6 ± 3.2	1.6 ± 2.0	44.4	74.2	6	n.t.	n.t.	–	–	–
YC × YC	10.7 ± 4.2	8.0 ± 5.6	72.1	57.2	8	n.t.	n.t.	–	–	–
JY × SFB	–	–	–	–	–	–	–	–	–	–
JY × LC	7.5 ± 5.4	0.4 ± 1.0	63.5	94.9	2	4.4 ± 2.6	1.9 ± 1.1	63.4	69.8	5
JY × YC	9.6 ± 3.4	1.3 ± 2.0	71.1	88.1	5	10.0 ± 3.8	0.6 ± 1.1	71.2	94.3	3
SFB × JY	–	–	–	–	–	–	–	–	–	–
LC × JY	5.8 ± 3.0	1.0 ± 1.6	68.1	85.3	4	6.5 ± 3.2	1.0 ± 1.4	64.8	86.7	4
YC × JY	9.3 ± 4.7	2.3 ± 3.6	69.8	80.2	5	13.4 ± 5.3	1.2 ± 2.8	62.9	91.8	3

Cross (female × male)	F3 production* (offspring/female/day) by oviparously generated F2				
	cysts	nauplii	H% of cysts	% encystm	ovv. females
SFB × SFB	n.t.	n.t.	–	–	–
JY × JY	n.t.	n.t.	–	–	–
LC × LC	n.t.	n.t.	–	–	–
YC × YC	n.t.	n.t.	–	–	–
JY × SFB	–	–	–	–	–
JY × LC	4.1 ± 6.0	0.3 ± 1.3	59.3	93.2	1
JY × YC	4.9 ± 4.2	1.1 ± 2.2	65.2	81.7	2
SFB × JY	–	–	–	–	–
LC × JY	5.1 ± 6.0	1.7 ± 3.4	64.2	75.0	3
YC × JY	6.4 ± 5.9	1.1 ± 2.3	67.3	85.3	2

b) cross-breeding

Table 7 summarizes the results of the reciprocal crosses among Jingyu Lake (JY), Yuncheng (YC), Lagkor Co (LC) and SFB specimens, and shows the offspring (cysts or nauplii) per female per day for the successive generations, the hatching percentage of the cysts produced, the encystment rate, and the number of ovoviviparously reproducing females in each combination.

Infertility of crosses between JY and SFB was illustrated by the production of non-hatching cysts and the absence of ovoviviparous offspring.

JY showed cross-fertility with both YC and LC, at least until F3. F3 cysts produced by these crosses showed hatching in the range 59.3–67.3% (51.0–83.3% in Fl generation) and a few F2 females (out of 10 replicates) reproduced ovoviviparously. The hatching percentage of the LC × LC cysts was low (3.2% in Fl cysts, 44.4% in F2 cysts), but the hibernation method was more effective in breaking diapause in the other combinations of crosses. The encystment rate did not show any clear trend over the different generations and combinations.

Discussion

Reports about the biotic elements of salt lakes at high altitude (>1000 m) are restricted. The aquatic fauna of the saltpans ('salares') on the South American Andes Altiplano (in Bolivia, extending into Peru and Chile) has been inventorized (Bayly, 1993; Dejoux, 1993; Williams et al., 1995), and together with other crustaceans, *Artemia* has been reported at altitudes of about 4000 m.

Much less has been published in international scientific literature about saline lakes in the vast (semi-) arid area of mountain ranges and high plateaux in the heart of the Asian continent (Russian Federation, China and Central Asian republics). A bisexual *Artemia* is reported in a number of Pamir salt lakes, among which Sasykkul in Tajikistan (Egorov, 1998), where the thermal regime is influenced by the presence of underground wells, which prevent the temperature of the bottom layer to drop below 0 °C in winter (Akhrorov, 2002).

Multidisciplinary study, using up-to-date *Artemia* characterisation techniques, identified *Artemia tibetiana* from Lagkor Co, Tibet (Abatzopoulos et al., 1998, 2002). No long-term ecological study of this lake (or any other on the Plateau) and its brine shrimp population is available, but the low resistance of this species to a standard heat shock test (as compared to strains from less extreme climates; Clegg et al., 2001) is explained as an adaptation to its environment. Another feature of the new species is the large size of its cysts (323.0–330.0 μm), larval (667 μm for instar I nauplii) and adult stage (Abatzopoulos et al., 1998).

The diameter of *A. tibetiana* cysts is nearly equalled by the sample from Jingyu Lake (320.0 μm). The Bozi Co population showed the smallest cyst size (284.5 μm), but this value is still in the range of the biggest parthenogenetic cysts reported, e.g. Margherita di Savoia (Italy) and Tuticorin (India), namely 280–285 μm, and well above values for current commercial samples like Great Salt Lake, 240–245 μm (Vanhaecke & Sorgeloos, 1980). Whatever the species status of the studied samples, the cysts are (very) big, especially compared to the other bisexual species from continental China, *A. sinica* from Yuncheng: 232 μm (Cai, 1989). This big cyst size has further been confirmed for new samples taken from four other Tibetan lakes, revealing a diameter in the range 291.0–358.1 μm (Yu et al., pers. comm.). Though a lot of other factors interfere (e.g. polyploid parthenogenetic strains are usually bigger sized than bisexuals) organisms in colder climates tend to be bigger than their counterparts at lower latitudes ('Bergmann's Rule'; Atkinson & Sibly, 1997). This was shown for freshwater copepods and cladocerans (Villalobos & Zuñiga, 1991; Gillooly & Dodson, 2000), rotifers (Stelzer, 2002), and perhaps is also illustrated by the size of the *Artemia* populations along a gradient of 20–50 ° south latitude on the South American Pacific coast (Gajardo et al., 1998).

The average instar I naupliar length (ranging between 540.2 and 607.1 μm, values for Haiyan and Jingyu Lake, respectively), though bigger than in most other strains (517 μm for Margherita di Savoia; Vanhaecke & Sorgeloos, 1980), is still far below the *A. tibetiana* value (667 μm; Abatzopoulos et al., 1998). No systematic research was done on the length of the adult animals.

Within the experimental conditions the cyst samples from the Qinghai-Tibet plateau showed higher hatching and survival at lower temperatures than the control strains from San Francisco Bay and Great Salt Lake. Despite this general pattern, the different strains didn't show an identical cold tolerance and/or preference, as is illustrated by the hatching pattern of Co Qen strain (Fig. 1) and the tolerance to heat shock of Co Qen and Jingyu Lake strains, as compared to Lagkor Co strain (Fig. 2). No *Artemia* is found in areas where year-round prevailing extremely low temperatures preclude its development (Persoone & Sorgeloos, 1980), but a lot of strains are found in the continental areas of North America and Asia with extremely cold winter temperatures, but where high summer temperatures allow cyst hatching and subsequent colonization of the environment. *A. tibetiana* survives in a habitat with annual temperatures fluctuating between –26 and +24 °C, and with an average annual air temperature of ±1.6 °C (Zheng, 1997). For strains from less extreme climates hatching, growth and maturation are delayed below the range 25–30 °C, as shown by the control strains (GSL, SFB) in our tests; the exact temperature sensitivity however is strain-dependent (Reeve, 1963; Von Hentig, 1971; Vanhaecke et al., 1984; Thoeye et al. 1987; Browne et al., 1988; Vanhaecke & Sorgeloos, 1989).

Artemia species have been the subject of numerous salinity studies (Croghan, 1958a, b; Bowen et al., 1985; D'Agostino & Provasoli, 1986; Triantaphyllidis et al., 1995; Abatzopoulos et al., 2003), revealing population-specific physiological tolerances to salinities, specific ions and ionic ratios. *Artemia* can withstand environments in which the ratio of the major anions and cations may be totally different from that in seawater (Cole & Brown, 1967; Persoone & Sorgeloos, 1980; Bowen et al., 1988). Since the osmotic pressure differs in function of the salt composition, the costs of osmoregulation also differ in media of the same salinity but with various ionic environments. In our study the Lagkor Co and Tibet A populations performed better in the carbonate and sulfate enriched artificial Lagkor Co medium. The ionic composition of the habitat can result in ecological isolation of particular *Artemia* strains, as illustrated for *A. franciscana* (Bowen et al., 1985; 1988). In comparative tests (e.g. in our cross-breeding tests) the ionic composition of the common culture medium may therefore interfere with our results as the salt composition may not be optimal for all strains tested.

The high contents in HUFA's, and mainly EPA, is a recurring characteristic in Tibetan *Artemia*. EPA values in the range 19.2–46.6 mg.g^{-1} dry weight have also been reported for cysts from four newly sampled lakes from this area (Yu et al., pers. comm.), which adds to the aquaculture potential of these strains, if size is not prohibitive. The HUFA profile of *Artemia* cysts reflects the feeding environment of the female parentals. Zheng (1997) reports *Dunaliella salina* and, to a lower extent, *Chlamydomonas* sp. as the main component of the phytoplankton flora in the saline lakes on the Qinghai-Tibet Plateau. *D. salina* has a high adaptation capacity to low temperature and to variable ionic composition, and is rich in proteins and β-carotene (Zheng, 1997). No data are given on the HUFA profile of these algae in the local conditions. In this respect, there may be a link between the HUFA pattern of the phytoplankton and the increased UV radiation at high altitudes. UV may affect virtually every aspect of life (survival, growth, reproduction, egg hatching, sex ratio) but effects may be very different between species and/or taxonomic groups (Häder et al., 1998; Sommaruga, 2001). The net effect on the food web may be extremely complex as all trophic levels are differently affected by UV, as shown in mesocosm and *in situ* enclosure experiments (Cabrera et al., 1997; Halac et al., 1997; Sommaruga et al., 1999). Generally bacterioplankton is affected to a greater extent than algae. The latter may thus be in a competitive advantage for nutrients, having an effect on their fatty acid contents (Plante & Arts, 2000). Other authors reported increased photodegradation of dissolved organic carbon as an effect of UV radiation, stimulating the food web (De Lange et al., 2003). Wangberg et al. (1999) found increased fatty acid content in marine phytoplankton at increased UV radiation levels.

The species status of the studied populations is not entirely clarified by our experiments. They all contained – to a variable degree – relatively high numbers of males and also parthenogenetically reproducing females. The sex ratio in the field, however, may differ from our laboratory data, due to possible selection during hatching and subsequent culture. Even in the field considerable seasonal fluctuations occur (Van Stappen et al., 2001). As these lakes have never been harvested systematically, the samples may be a mixture of cysts produced in different areas of the lake, spread over several seasons or years. The presence of parthenogenetic females in all samples complicates the determination of the species status of the bisexual individuals. Although this presence was minimal in the Jingyu Lake sample, our results do not allow confirmation of this bisexual sample as either *A. tibetiana* or *A. sinica*. Production of fertile offspring between individuals belonging to different 'Eastern Old World' *Artemia* bisexual species is not uncommon: Pilla & Beardmore (1994) successfully crossed *A. sinica* with *A. urmiana* and *Artemia* sp. from Kazakhstan, with no apparent hybrid breakdown at later generations (up to F3). Between *A. tibetiana* and *A. sinica*, partial fertility through F2 and F3 has been shown as well (Abatzopoulos et al., 2002). Cross-breeding through more successive generations might shed some more light on the species status of the Jingyu and other samples. Additionally, testing of alternative diapause deactivation methods on cysts produced in cross-breeding tests may result in higher hatching: the low values obtained for LC × LC cysts (3.2% in F1, 44.4% in F2) suggests that the standard incubation of the cysts at –18 °C for two weeks was insufficient for optimal diapause breaking.

Though examples of natural coexistence of different Asian bisexual species (*A. urmiana*, *A. sinica* and *A. tibetiana*) are not known, there is evidence for coexistence of bisexual species with parthenogenetic populations, and for coexistence of different parthenogenetic strains, e.g. in Spain (Amat, 1980; 1983; Amat et al., 1995). Temporal cycling or niche partitioning may be the result of different relative fitness of the coexisting strains to the temperature profile of the environment (Browne, 1980; Browne et al., 1988; Browne & Halanych, 1989). Bowen et al. (1978) reported that parthenogenetic strains have more haemoglobin than sexual species, which might be advantageous at high salt concentration and at high altitudes. Partial coexistence has also been reported in the area of Lake Urmia, Iran, where there is evidence that the lake itself is the habitat of the bisexual species *A. urmiana* and a smaller fraction of parthenogenetic individuals, whereas in the adjacent lagoons and salt ponds with very different conditions of temperature and salinity only the parthenogenetic population is found (Van Stappen, 2002).

Techniques of DNA fingerprinting can result in a breakthrough in the understanding of genetic relationships between different populations and in the problem of coexistence of strains. In a database of 65 *Artemia* samples, based on RFLP patterns of a mitochondrial rDNA fragment (Wang et al., 2003), *A. tibetiana* from Lagkor Co clusters together with the samples from Tibet A and Bozi Co. The samples from Co Qen and Jingyu Lake, however, appear in this dendrogram in a large cluster of parthenogenetic and unidentified populations. All Tibetan samples show a large genetic distance from the *A. sinica* samples in this database. The high degree of diversity within each cluster, even among samples from the same habitat, illustrates that future analyses should focus on individuals, rather than on batches of cysts (Wang et al., 2003).

Conclusions

The *Artemia* biodiversity of PR China shows a complex pattern. The prevailing mode of reproduction in the coastal habitats in China is parthenogenesis (Xin et al., 1994), though in recent years some populations are mixed with, or have been outcompeted by, introduced *A. franciscana*. Numerous parthenogenetic populations also exist in inland lakes, but also bisexual populations are found in inland China, probably belonging to the species *A. sinica* (Van Stappen, 2002). The population of Lagkor Co, Tibet, has been identified as *A. tibetiana* (Abatzopoulos et al., 1998, 2002). Based on the available samples, our experiments show that bisexuals are also found in other lakes on the Qinghai-Tibet plateau, co-occurring with parthenogenetic populations. These populations share, to a variable degree, common characteristics like large cyst size, high HUFA content and tolerance to low temperatures. DNA fingerprinting techniques should bring decisive evidence on their exact species status and on the mixed nature of the populations.

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Volumetric and aerial rates of heterotrophic bacterial production in epi- and hypolimnia: the role of nutrients and system morphometry

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Abstract

Epilimnetic and hypolimnetic bacterial production (BP) were measured once in summer, by the incorporation of [^{3}H] - Leucine in each of 14 Quebec (Canada) lakes varying in nutrient content and morphometry. The epilimnetic and hypolimnetic BP were evaluated at two scales: the common per unit volume and areal (m^{-2}) scale. The per unit volume scale epilimnetic BP was best predicted by total phosphorus (TP, $r^2 = 0.63$), and by water residence time (WRT $r^2 = 0.57$), with WRT serving as a surrogate for the nutrient and organic matter supply from the catchments. Total phosphorus and lake mean depth (Z_m) together explained 79% of the variation in epilimnetic BP (l^{-1}). In contrast, hypolimnetic BP (l^{-1}) was neither linked to nutrients (TP or TN) or dissolved organic carbon (DOC) but only to measures of lake morphometry and best of all to hypolimnetic thickness (Zh; $r^2 = 0.74$). With increased Zh, there is an increased dilution of settling organic particles and their nutrients, resulting in a decrease in BP per litre. Conversely, when BP is expressed in areal units (m^{-2}), hypolimnetic production increases with increasing hypolimnetic thickness. Water column thickness is a master variable, which together with Chl *a* (abundance of particles) determines hypolimnetic BP at the whole system scale even though the trophic status is the best single indicator of epilimnetic BP on a volumetric scale. Conclusions drawn invariably change with the scale of investigation. Moreover, it is clear that lake morphometry has a major impact on BP. A comparison of whole water column integrated BP with literature derived estimates of the equivalent sediment production (m^{-2}) below suggests that if the estimated sediment rates are not complete technique artefacts, they are likely to be an order of magnitude higher than the water column rates (m^{-2}) at the maximum depth sampling sites. The relative importance of the sediments could be expected to rise with a decline in the maximum depth of lakes, characterized by progressively thinner hypolimnia. The present findings point to both a primarily allocthonous fuelling of sediment production and an uncoupling of water and sediment BP.

Introduction

High bacterial carbon demands have shown that detrital food webs play a central role in the cycling of carbon in the pelagic zone of lakes (Cole et al., 1988; del Giorgio et al., 1997). Decomposition processes appear to dominate planktonic metabolism, and as a consequence, net heterotophy seems to be a widespread phenomenon (Smith & Hollibaugh, 1993; Cole et al., 1994; del Giorgio et al., 1997). Bacterial biomass production (BP) per unit area (μg C m^{-2} d^{-1}) appears to average about 30% of planktonic net primary production (PP), in fresh and marine waters (Cole et al., 1988). However, BP can exceed phytoplankton productivity in oligotrophic waters and in humic lakes (e.g. Jonsson et al., 2001).

A high BP:PP ratio implies that the planktonic heterotrophic bacteria convert autochthonous produced dissolved organic carbon (DOC) to particulate organic carbon (POC) with a high efficiency and/or complement this carbon source with organic matter derived from their catchments and littoral zones There is evidence for both (e.g. Jonsson et al., 2001). Regardless, BP estimates are fundamental to the investigation of carbon flows and important in the assessment of aquatic secondary productivity, particularly in lakes, where the bacteria:phytoplankton biomass ratio is typically higher than in the oceans (Simon et al., 1992). In addition BP is important in nitrogen and phosphorus cycling. Bacteria not only have a high nutrient to carbon ratio (e.g., Fagerbakke et al., 1996), but also an exceptionally efficient nutrient uptake (Azam et al., 1983), that allows them to compete favourably with the phytoplankton for inorganic nitrogen in marine and for inorganic phosphorus in oligotrophic freshwater (e.g., Currie & Kalff 1984).

Among system comparisons along gradients of nutrient enrichment have provided important insights into the factors controlling planktonic bacterial populations, directly or indirectly. Bacterial abundance (l^{-1}) and production increases at a slower rate than Chl *a*, (Biddanda et al., 2001) with increasing trophic status, whereas BP (l^{-1}) increases at roughly the same rate as bacterial abundance (Cole et al., 1988; White et al., 1991) and PP (Cole et al., 1988). However, patterns are much weaker in freshwater than marine systems, indicating the relevance of yet other variables in explaining BP in freshwater. Allochthonous organic carbon inputs presumably contribute importantly to the high variability between bacteria and algal biomass relationships in inland waters. However, nutrients such as phosphorus and nitrogen may represent freshwater bacterial resources better (Cimbleris & Kalff, 1998) than organic matter (algal biomass), as shown by stronger among lake correlations between bacterial abundance and total phosphorus concentration (TP) than between bacterial abundance and Chl *a* (Currie, 1990). Thus, bacterioplankton growth in inland waters may at times be more directly constrained by a shortage of dissolved inorganic P or N rather than by the availability of organic substrates.

With the emphasis in planktonic bacterial ecology on within system substrate and nutrient availability for growth, and more recently on the importance of predation in constraining bacterial and community production per unit volume (l^{-1}), the potential importance of catchment and lake morphometry in affecting BP has been neglected. But Currie (1990) in an among lake study noted epilimnetic BP (l^{-1}) to decline with increasing lake surface area and mean depth. Equally serious, the literature on planktonic heterotrophic bacteria has overwhelmingly examined their abundance and activity on a volume basis (l^{-1}) and in epilimnia only. The volumetric focus is not surprising because microbial ecology, with its roots in the physiological studies of laboratory populations, has been preoccupied with the elucidation of proximal causes for changes in community growth rates in the plankton, examined in flasks (l^{-1}). The proximal focus has meant that there has been little interest in more distal causes, such as the impact of land-use, system flushing, and lake morphometry on bacterial metabolism, nor on bacterial metabolism at the whole system scale, approximated by expressing findings on an aerial basis (m^{-2}).

Here we combine measures of the impact of environmental factors on planktonic bacterial production at both the traditional volumetric scale (l^{-1}) with those expressed at the aerial scale (m^{-2}), to demonstrate the importance of system morphometry on BP, and secondly to show the large, but generally overlooked, impact of the spatial scale selected on the conclusions drawn.

Methods

Sampling

Fourteen lakes, in the Eastern Townships and Southern Laurentians of Quebec (about 45° N and 72° W) varying in trophic status, DOC content and morphometry, where each sampled once during the summer of 1995. Integrated epilimnetic water samples were taking with a 3.5 cm diameter plastic tube from 1 m above the thermocline to the surface. Hypolimnetic water was collected with a Van Dorn bottle, half way between the bottom of the thermocline and the lake bottom, at sites close to the maximum depths. The samples were stored in 20-l dark collapsible plastic containers and maintained close to the *in situ* temperature until the start of the incubations 3–4 h later. As sampling was restricted to summer, water temperatures among the lakes located not far from each other differed relatively little, with epilimnetic and hypolimnetic waters ranging from 20 to 26 °C and from 7 to 13 °C, respectively. Consequently, the temperature in the 12 lakes that stratified differed much more between individual epilimnia and hypolimnia than among system.

[^{3}H] - leucine incorporation and bacterial production

BP was determined from measurements of [^{3}H] - leucine incorporation rates (Kirchman et al., 1985), using the centrifugation method developed by Smith & Azam (1992). L-[2,3,4,5, -^{3}H] leucine (specific activity = 3.07 Tbq mmol^{-1} - ICN radioactive chemicals) was added into 1.5 ml microcentrifuge tubes (Sarstedt) containing 1.3 ml of water sample to produce a final concentration of 41 nM (the first five lakes were analyzed using [^{3}H] - leucine with a different specific activity: 4.48 Tbq mmol^{-1}). The risk of extra cellular isotope dilution in eutrophic waters (Jørgensen, 1992) was reduced by adding leucine at 41 nM, a concentration low enough to not saturate bacterial incorporation rates and to be take up by bacteria only (Smith & Azam, 1992). For the first eight lakes, three blanks consisting of time-zero controls and triplicate epilimnetic and hypolimnetic water samples were incubated for 20 and 40 min, using a water bath at *in situ* temperature. The 20-min period was adopted for the final six lakes, using four replicates for blanks and samples. Incubations were terminated upon the addition of 70:1 of 100% (w/v) trichloroacetic acid (TCA) (5% final concentration). The microcentrifuge tubes were centrifuged for ten minutes at 16 000 × *g* and aspirated. Each sample was washed following the addition of 1.37 ml 5% TCA and vortex mixing. The tubes were once more centrifuged and aspirated. Next, 0.4 ml of liquid scintillation cocktail (Universol – ICN Radioactive Chemicals) was added per tube, followed by vortexing. The tubes were placed into scintillation vials, and radioassayed in a liquid scintillation counter using the samples channel ratio method and quenched tritium standard calibration curves. The coefficients of variation averaged 18% for both epilimnetic and hypolimnetic samples. [^{3}H] – Leucine incorporation rates were calculated according to Bell (1993), using a formula originally developed for [^{3}H] – thymidine. The rates provide measurements of bacterial protein synthesis (Kirchman et al., 1985), which can be directly translated into bacterial carbon production following Simon & Azam (1989). An intracellular isotope dilution of 2 was assumed.

Analyses of trophic and morphometric characteristics

Information on lake morphometry and trophic status was obtained from bathymetric maps and the literature (Schallenberg & Kalff, 1993; del Giorgio & Peters, 1994). Nutrient concentrations were determined spectrophotometrically, using three replicates per analysis. TN and TP were analyzed according to APHA (1989) and Griesbach & Peters (1991), respectively. High temperature combustion (Shimadzu TOC-5050 analyser) was initially used for DOC, but as concentrations so determined were closely correlated with water colour, most measurements were made spectrophotometrically (Cuthbert & del Giorgio, 1992), followed by conversion to DOC units (mg l^{-1}) according to Rasmussen et al. (1989). Chlorophyll concentrations were obtained from Schallenberg (1992) and del Giorgio & Peters (1993), while bacterial abundance data for the lakes were derived from del Giorgio and Peters (1993).

Data analysis

All data were log-transformed to equalize the variance and attain homoscedasticiy. Bacterial production was related to resource attributes and lake morphometry by correlation and least-squares regression analyses using SAS (1987) and SYSTAT (1996). Geometric mean regressions (model II) were also determined (Sokal & Rohlf, 1995) to account for the possibility that the regression coefficients (slopes) could be underestimated due to errors in the independent variables.

Results

Epilimnetic bacterial production (BP) per unit volume (μg C l^{-1} d^{-1}) increases with lake trophic status, best predicted by TP (Fig. 1), the element in the lowest concentration and with the largest among lake variation in both the epilimnia and hypolimnia (Table 1). The slopes of the lines linking epilimnetic BP with both TP and chlorophyll *a* (Chl *a*) are much lower than one, showing that per unit increase in TP or Chl *a* there is a progressively smaller increase in BP (Table 2). The epilimnetic BP per unit volume (l^{-1}) was, furthermore, well coupled with lake morphometric variables, decreasing as epilimnetic thickness increases and increasing as the catchment area (CA) to lake area (LA) ratio rises (Table 3). Epilimnetic BP (l^{-1}) rises as water retention time (WRT or τ_w) declines, with more than half the variation in BP explained by WRT alone (Fig. 2). The variance explained rises to nearly 80% when both trophic (TP or TN) and morphometric variables (lake mean depth (Zm) or WRT) are considered (Table 2). In sharp contrast, the per unit area epilimnetic BP (μg C m $^{-2}$ d^{-1}) was not related to any of

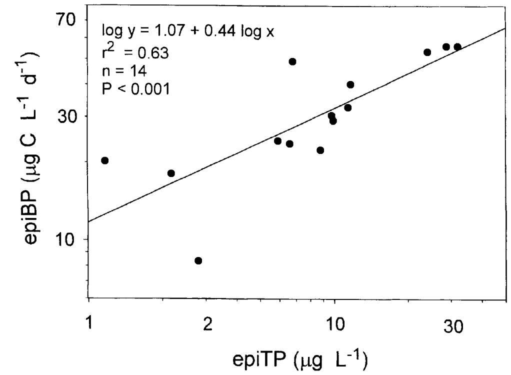

Figure 1. Epilimnetic bacterial production per unit volume (µg C l^{-1} d^{-1}) as a function of epilmnetic total phosphorus (epi TP; µg l^{-1}) among 14 Southern Quebec lakes.

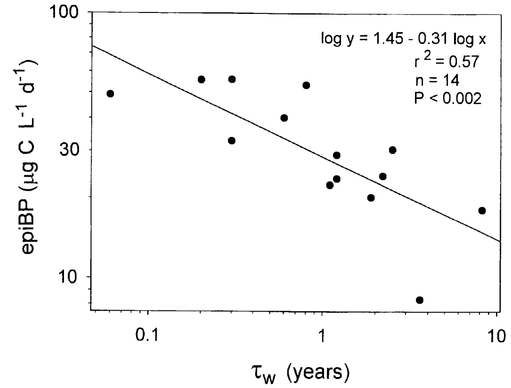

Figure 2. Epilimnetic bacterial production per unit volume (µg C l^{-1} d^{-1}) as a function of water retention time (τ_w) among 14 Southern Quebec lakes.

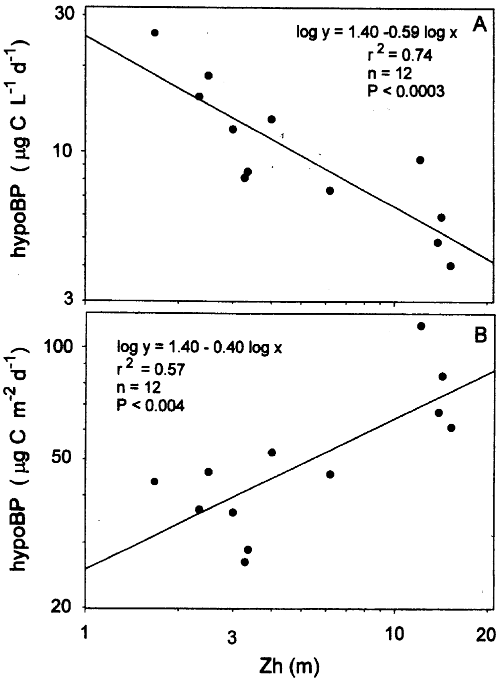

Figure 3. (A) Volumetric (µg C l^{-1} d^{-1}) and (B) areal (µg C m^{-2} d^{-1}) hypolimnetic bacterial production as a function of mean hypolimnetic thickness (Zh) among 12 Southern Quebec lakes.

the trophic and/or morphometric variables mentioned above (Table 3).

Hypolimnetic BP (l^{-1}) was, in contrast to the epilimnetic BP (l^{-1}), not a function of hypolimnetic nutrient, nor even of epilimnetic of algal biomass (Chl *a*) or hypolimnetic DOC but only linked to aspects of lake morphometry (Table 4). Hypolimnetic BP (l^{-1}) declines with increasing hypolimnetic thickness (Fig. 3A), which alone accounts for 74% of the among lake variation in BP. The opposite pattern emerges when BP is expressed on an aerial basis (m^{-2}), which shows BP to increase with hypolimnetic thickness (Zh) (Fig. 3B). Intriguingly, while hypolimnetic BP (l^{-1}) was unaffected by epilimnetic Chl *a* concentrations, the hypolimnetic BP (m^{-2}) declines as epilimnetic Chl *a* concentrations increase, with the Chl *a* explaining about half the variation in BP (m^{-2}) (Table 4).

Discussion

Epilimnetic production per unit volume as a function of trophic variables and system morphometry

The finding that BP (l^{-1}) increases along a trophic gradient (Fig. 1, Table 1) is not novel but provides and an always useful confirmation of studies else where (see Kalff, 2001). TP and its correlate TN, are clearly better indicators of BP than the uncorrelated DOC concentrations (Table 3). The importance of TP appears, as in the phytoplankton (Peters & Bergmann, 1982), to be the result of TP being a much better indicator of available phosphorus over the longer term and among systems than DOC is of the available dissolved organic carbon pool. In support of this Cammack (2002) and Cammack et al. (in preparation), working

Table 1. Mean bacterial production (BP) per unit volume (l^{-1}) and mean concentrations of dissolved organic carbon (DOC; mg l^{-1}), total nitrogen (TN; μg l^{-1}), and total phosphorus (TP; μg l^{-1}), in the epilimnio (epi) and hypolimnia (hypo), across 14 Southern Quebec Lakes (Y: Yamasaka; W: Waterloo; B: Brome; D: D'Argent; CI: Coulombe; SF: St-Francois; Bp: Brompton; T: Truite; Cr: Cromwell; Co: Connelly; L: Loverling; O: Orford; N: Nicolet; Cc: Croche). BP Values show the standard deviations

Lakes	epiBP	epiDOC	epiTN	epiTP	hypoBP	hypoDOC	hypoTN	hypoTP
Y	56 ± 34	5.7	828	29	8 ± 3.0	5.4	1012	43
W	56 ± 20	5.0	719	32	–	–	–	–
B	54 ±3.9	3.2	636	24	–	–	–	
D	40 ±1.3	4.7	608	12	19 ± 1.8	6.4	559	33
Cl	33 ± 8.8	9.9	628	11	12 ± 1.4	8.9	479	11
SF	29 ± 5.3	9.6	835	10	9 ± 1.5	8.8	908	11
Bp	30 ± 2.5	5.5	674	10	5 ± 0.1	5.6	642	10
T	22 ± 4.3	5.0	761	9	9 ± 3.4	5.1	820	12
Cr	49 ± 0.9	8.0	313	7	16 ± 2.7	13.5	928	16
Co	24 ± 3.7	4.6	461	7	7 ± 1.6	4.4	658	10
L	24 ± 4.3	4.3	692	6	13 ± 2.3	4.2	365	11
O	8 ± 1.8	3.0	149	3	6 ±1.4	3.0	174	3
N	18 ± 1.6	3.0	311	2	4 ± 0.4	3.2	430	2
Cc	20 ± 3.0	5.5	382	1	26 ± 2.1	5.5	500	7

Table 2. Simple and multivariate linear regressions for the relationships between trophic and morphometric variables and between productions (BP) per unit volume in the epilimnia of 14 Quebec lakes. The models are described by the equations log (y) = a +b * log (x) and log (y) = a + b_1 * log (x_1) + b2 * log (x_2), where y = dependent variable; x = independent variable; a = intercept and b = slope. The coefficient of determination (r^2), the standard error of the estimate (SEE) and the significance level (*P*) are given. The simple regressions present the parameters for both, ordinary least - squares (1) and geometric mean (2) models. BA: epilimnetic bacterial abundance (X 10^6 cells ml^{-1}); Chl *a*: epilimnetic chlorophyll *a* (μg l^{-1}); τ_w: water retention time (years); Zm: lake mean depth (m). Other abbreviations as in Table 1

y	x	slope	intercept	r^2	SEE	*P*
epiBP l^{-1}	epiTP	0.44 ± 0.10 (1)	1.07 ± 0.10 (1)	0.63	0.147	<0.001
		0.56 ± 0.10 (2)	0.96 ± 0.10 (2)			
epiBP l^{-1}	Chl *a*	0.39 ± 0.11 (1)	1.24 ± 0.08 (1)	0.52	0.166	<0.003
		0.54 ± 0.11 (2)	1.15 ± 0.08 (2)			
Chl *a*	epiTP	0.68 ± 0.22 (1)	−0.04 ± 0.22 (1)	0.44	0.333	<0.010
		1.03 ± 0.22 (2)	−0.35 ± 0.22 (2)			
BA	epiTP	0.41 ± 0.07 (1)	0.15 ± 0.07 (1)	0.74	0.105	<0.0001
		0.48 ± 0.07 (2)	0.09 ± 0.07 (2)			
epiBP l^{-1}	τw	− 0.31 ± 0.08 (1)	1.45 ±0.04 (1)	0.57	0.159	<0.002
		− 0.41 ± 0.08 (2)	1.49 ± 0.04 (2)			
epiBP l^{-1}	ZM	−0.55 ± 0.19 (1)	1.94 ± 0.17 (1)	0.42	0.183	<0.012
		−0.85 ± 0.19 (2)	0.74 ± 0.17 (2)			
epiBP l^{-1}	epiTP	0.36 ± 0.08 (1)	1.45 ± 0.15 (1)	0.79	0.115	<0.0001
	Zm	−0.37 ± 0.12 (1)				
epiBP l^{-1}	epiTN	0.52 ± 0.16 (1)	0.03 ± 0.44 (1)	0.78	0.118	<0.0001
	τw	−0.25 ± 0.06 (1)				

Table 3. Pearson correlation matrix of trophic and morphometric lake variables in relation to volumetric (epiBP l^{-1}; μg C liter $^{-1}$ d^{-1}) and areal (epiBP m^{-2}; μg C m^{-2} d^{-1}) bacterial production in the epilimnia of the southern Quebec lakes. Ze: epilimnetic thickness (m); CA / LA = drainage ratio (see text). Other abbreviations as in Tables 1 and 2. All variables were log transformed before analysis. $n = 14$ (except epi BP m^{-2} and Ze: $n = 12$)

	epiBP l^{-1}	epiBP m^{-2}	Chl *a*	BA	epiDOC	epiTN	epiTP	Zm	Ze	τ_W	CA/LA
epiBP l^{-1}	1										
epiBP m^{-2}	0.68*	1									
Chl *a*	0.72*	0.17	1								
BA	0.48	0.11	0.54*	1							
epiDOC	0.38	0.45	0.18	−0.07	1						
epiTN	0.66**	0.50	0.46	0.49	0.43	1					
epiTP	0.79***	0.36	0.66**	0.86***	0.23	0.69**	1				
Am	−0.65*	−0.03	−0.74**	−0.06	−0.31	−0.27	−0.34	1			
Ze	−0.60*	0.18	−0.71**	−0.29	−0.36	−0.33	−0.56	0.76**	1		
τ_W	−0.75**	−0.23	−0.66**	−0.29	−0.59*	−0.29	−0.59*	0.75**	0.79**	1	
CA/LA	0.60*	0.31	0.45	0.31	0.65*	0.18	0.55*	−0.42	−0.52	−0.89	1

***$P < 0.001$ **$P < 0.01$ *$P < 0.05$; all other correlations are non significant ($P > 0.05$)

in the same region, report a particular group of fluorescing organic compounds to be a much better predictor of BP in nature than the DOC pool as whole. However, the concentration range over which otherwise important environmental factors are examined is an important determinant of whether their impact on the biota can be detected against a background of much environmental variation. Epilimnetic TP concentrations is in the present study ranged 30 fold, whereas among lake DOC concentrations ranged only 3 fold, thereby greatly reducing the possibility of detecting a DOC impact.

Not surprisingly, larger drainage basins in any one area release more nutrients and DOC than smaller counterparts exhibiting a similar land use (Kalff, 2001). This is reflected in a higher epilimnetic BP in lakes with a large catchment area to lake area ratio (CA/LA, Table 3). Even so, resource supply to the planktonic bacteria is better represented by the average hydraulic water retention time (WRT or τ_W, yr), which is determined in part by the size of the catchment area available to capture precipitation and to export nutrients and organic carbon, and in part by the lake volume receiving the inputs, with the easy to determine lake area only a rough proxy for the volume. A longer WRT implies a relatively smaller catchment input of nutrients and DOC, an increased system retention (reduced flushing) of allochthonously and authochthonously produced particles, and thereby an increased possibility of particles leaving the epilimnion for the hypolimnia and sediments rather than being flushed from the system. The easily computed average WRT predict epilimnetic BP (l^{-1}) about as well as its correlate TP (Figs 1 and 2), showing BP to rise as the WRT declines and the associated nutrient and DOC loadings increase (Table 3). However, BP is much better predicted by considering not only nutrient concentrations but also one of two physical factors (WRT or Zm), with the nutrient - morphometry combination explaining nearly 80% of the variation in BP (l^{-1}) (Table 2).

Lake morphometry: the master variable in predicting hypolimnetic production

In contrast to the epilimnia where both nutrients and algal biomass are closely linked to BP (l^{-1}), the hypolimnetic BP (l^{-1}) is coupled only to measures of lake morphometry (Table 4). The negative relationships observed between water column thickness (Ze and its correlate Zm) and BP (l^{-1}) in the epilimnia (Table 3), is even more strongly evident in the hypolimnia. However, contrary to a relatively modest among system difference in lake area, fetch, and the resulting epilimnetic thickness (Ze) (see Kalff, 2001), the differences in hypolimnetic thickness (Zh, a surrogate for the volume), are large enough to overwhelm the impact of smaller differences in nutrient and substrate supply obtained from the overlying epilimnia, preventing a detection of their influence on volumetric BP. Consequently, measures of lake morphometry (Zm, Zh), emerge as the only predictors of hypolimnetic BP (l^{-1}) (Table 4). That the principal predictor

Table 4. Pearson correlation matrix of variables related to the hypolimnia of 12 Quebec lakes. Bacterial production is expressed both per unit volume (hypoBP l^{-1}, μg C liter $^{-1}$ d^{-1}) and area (hypoBP m^{-2}; μg C m^{-2} d^{-1}). Whole water column (epilimnion + hypolimnion) bacterial production (wcolBP l^{-1}; wcolBP m^{-2}) is also shown. LA = lake area (km^{-2}); Zh = mean hypolimnetic thickness (m). Other abbreviations in Tables 1, 2, and 3. All variables were log transformed before analysis

	Hypo BP l^{-1}	Hypo BP m^{-2}	Wcol BP l^{-1}	Wcol BP m^{-2}	Chl *a*	Hypo DOC	Hypo TN	Hypo TP	LA	Zm	Ze	Zh
hypoBP l^{-1}	1											
hypoBP m^{-2}	−0.32	1										
wcolBP l^{-1}	0.65*	−0.53	1									
wcolBP m^{-2}	−0.26	0.60*	0.11	1								
Chl *a*	0.44	−0.70**	0.72**	−0.24	1							
hypoDOC	0.52	−0.20	0.75**	0.29	0.42	1						
hypoTN	0.15	−0.40	0.71**	0.18	0.55	0.64*	1					
hypoTP	0.45	−0.52	0.83***	−0.08	0.76**	0.54	0.64*	1				
LA	−0.59*	−0.63*	−0.27	0.66*	−0.17	−0.21	0.01	−0.08	1			
Zm	−0.79**	0.77**	−0.78	0.43	−0.73**	−0.59*	−0.44	−0.61*	0.63*	1		
Ze	−0.51	0.71**	−0.68*	0.51	−0.71**	−0.43	−0.49	−0.83***	0.45	0.76**	1	
Zh	−0.86***	0.76**	−0.73**	0.50	−0.68*	−0.47	−0.32	−0.59*	0.75**	0.96***	0.73**	1

$^{***}P < 0.001$ $^{**}P < 0.01$ $^{*}P < 0.05$; all other correlations are non significant ($P > 0.05$)

of BP changes with changes in the spatial scale (epi- vs. hypolimnion) is further evident when instead the water column as a whole (Wcol BP l^{-1}) is considered. Over that spatial scale, the among system variation in epilimnetic Chl *a* is sufficient to allow both Chl *a* and morphometry to serve on predictors of BP (Table 4). The decline in BP (l^{-1}) with increasing hypolimnetic thickness (Zh, Fig. 3A) and its surrogate mean depth (Zm, Table 4), reflects an increased dilution of sedimenting organic particles and associated nitrogen and phosphorus. The decline in volumetric production with increasing hypolimnetic thickness (Fig. 3A) is supported by studies on hypolimnetic respiration as a function of lake morphometry (Charlton, 1980; Cornett & Rigler, 1980) that show, as we do here for BP (l^{-1}), that thicker hypolimnia exhibit lower rates of dissolved oxygen (DO) consumption (respiration). This is interpreted to be the result of an increased dilution of sedimenting epilimnetic particles in larger volumes. The dilution effect represented by Zh and Zm is sufficiently large to obscure the effect of among system difference in the supply of algal particles and concentration of DO, as well as TN or TP (Table 4).

Both Hypolimnetic BP per unit area (m^{-2}) and areal DO consumption (Cornett & Rigler, 1980) increase with increasing hypolimnetic thickness (Zh, Fig. 3B) and its correlate mean depth (Zm, Table 4). This is interpreted to be the result of an increasingly long hypolimnetic transit time, allowing a more complete water column utilization of the available sedimenting particles. Zh alone explains nearly 60% of the variation in hypolimnetic BP (m^{-2}) (Fig. 3B). The counterintuitive observation of a higher BP (m^{-2}) in lakes characterized by lower epilimnetic levels of Chl *a* (Table 4) is the outcome of the overriding effect of hypolimnetic thickness (volume) and time available for sedimentation on BP (m^{-2}), with the impact sufficiently large to offset the effect of a relatively more modest difference on BP of the epilimnetic algal biomass (Chl *a*) and its availability for sedimentation. In other words, the effect on BP on a long hypolimnetic particle transit time in the typically thicker hypolimnia of less eutrophic lakes is much larger than the positive effect of a larger number of sedimenting particles in more eutrophic water.

Pelagic versus *sediment production: a speculative exploration*

The lower BP per litre and the slowly increasing BP (m^{-2}) with increasing hypolimnetic thickness (Zh) point, respectively, to an increased dilution of sedimenting organic matter and an increasingly complete utilization in the water column of available particles (Fig 3). With increased time for water column metabolism in thicker hypolimnia (greater volume) fewer of the particles will reach the sediments for BP production and respiration there. This has been demonstrated for sediment respiration by den Heyer & Kalff (1998) who showed a systematic reduction (m^{-2}) with increasing depth in an overlapping set of lakes. The lack of a link between sediment bacterial abundance and epilimnetic Chl *a* (Schallenberg & Kalff, 1993) in an other overlapping set of local lakes fits the pattern in suggesting that the authochthonously produced, and presumably more available organic particles (Coffin et al., 1993; Jonsson et al., 2001), are largely consumed in the water column. If so, sediment BP (m^{-2}) should be a modest fraction of the water column production (m^{-2}), at least in the present lakes characterized by thick hypolimnia at the maximum depth sampling site.

Unfortunately, we were unable to measure sediment BP to test the hypothesis of disproportionately low sediment versus water column rates. However we explored the hypothesis, both by an examination of the small sediment BP literature and by making rough estimates of sediment BP based on a conversion of sediment respiration data (den Heyer & Kalff, 1998) to production rates. Local determinations of sediment BP (Gasol et al., 1993; Sanders et al., 1993), using ^{3}H –thymidine incorporation, point not to lower sediment rates but instead to rates (m^{-2}) much higher than the water column rates measured by the uptake of ^{3}H – leucine. The disproportionately high sediment rates, if not a technique artefact, point to an important role of allochthonous organic matter in fuelling sediment BP. That allochthonous organic matter plays an important role in sediment BP was evident in a humic Swedish lake where a mass-balance analysis showed about a third of the allochthonously derived sediment carbon to be metabolized annually. (Jonsson et al., 2001) Nevertheless, the reported sediment BP data (Gasol et al., 1993; Saunders et al., 1993) may be technique artefacts unable to withstand future scrutiny. The ^{3}H – thymidine technique, developed for use in aerobic water columns, may not provide a reliable measure of sediment production. There are suggestions that some bacteria do not take up thymidine under the hypoxic or anoxic conditions that characterize sediments (Sanders et al., 1993). If correct that would imply the apparently much higher sediment rates to be underestimates.

To further explore the striking suggestion that little of the whole system BP appears to occur in the water column, even at the maximum depth sampling sites, we computed independent estimates of sediment production based on the evolution (respiration) of CO_2 plus CH_4 (den Heyer & Kalff, 1998) to compare with our pelagic BP measurements. The validity of the comparison is diminished by not having an empirical sediment bacterial growth efficiency (BGE) to convert respiration to production rates. But regardless whether a high or low conversion efficiency is assumed, the computed sediment BP falls within the range obtained using thymidine uptake in local lakes (Gasol et al., 1993; Sander and Kalff, 1993). Next, assuming the sediment bacteria to have access to reasonably good quality organic matter in a nutrient rich sediment environment, by assigning a BGE of 20%, the maximum computed sediment BP (58 mg C m^{-2} d^{-1}) was close to the average planktonic BP rate (50 mg C m^{-2} d^{-1}), as measured using the thymidine method in the plankton of an overlapping set of lakes (Gasol et al. 1993). Assuming instead a much more likely BGE of only 2% for allochthonous organic matter of low quality (del Giorgio and Cole 1998) not consumed in the water column, generated as estimated BP of 2.1 mg C m^{-2} d^{-1} (Fig. 4), a value close to the lowest measured sediment rates in the literature. Even so, more than 90% of the computed BP would have occurred in the sediments at the maximum depth sampling sites. Although no single line of evidence for the proposed much higher sediment than water column BP (m^{-2}) is conclusive, together they suggest the sediment BP to be no lower but more likely to be an order of magnitude higher in the profundal sediments than in the overlying water column of the forest lakes, fuelled it appears by catchment and littoral zone derived organic matter. The disproportionately high estimated sediment contribution to BP (m^{-2}) obtained at the maximum depth of sampling would, by implication, be even higher in shallow lakes, characterized by thinner hypolimnia, and contribute close to 100% in lakes too shallow to allow a seasonal stratification, except after sediment and bacterial resuspension following high wind events (See Kalff, 2001). The present study, by sampling the lakes at the site of maximum depth, underestimates the sediment contribution from a whole lake perspective because lake morphometry typically resembles a saucer shape more so than the diagrammatic deep bowls shown in textbooks. Consequently, a large fraction of the sediments are overlain by a shallow epilimnetic water column, allowing sediments to dominate BP even more than is suggested by work at the stations of maximum depth.

Conclusions

A combination of simple trophic, morphometric and catchment variables (TP and Zm; TN and τ_w, CA/LA) were able to explain most of the variation in epi-

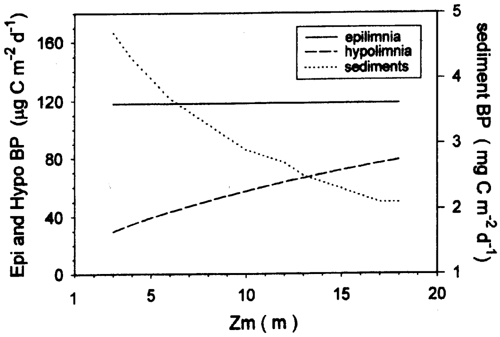

Figure 4. A partially conceptual model of areal bacterial production in lakes based on linear regression models having lake mean depth (Zm) as the predictor, and on the epilimnetic and hypolimnetic bacterial production. Sediment BP (mg C m^{-2} d^{-1}) was estimated from sediment respiration using the equation: areal sediment mineralization = 26 – 14log (Zm) (den Heyer & Kalff, 1998), and assuming a bacterial growth efficiency of 2%.

limnetic BP (l^{-1}). TP was the single best predictor of epilimnetic production. Hypolimnetic BP per unit volume changed systematically with depth, indicating a strong influence of substrate supply from the upper strata. Shallower lakes have disproportionately thin (small volume) hypolimnia and therefore, experience less dilution of incoming organic matter and associated nutrients, allowing higher BP (l^{-1}) than for their deeper counterparts. Conversely, thicker hypolimnia allow a more complete organic matter utilization in the water column during a longer transit time, allowing a higher areal rate of BP (m^{-2}). Morphometry is an important but largely overlooked determinant of BP and the 'causes' inferred clearly change with the spatial scale examined. A comparison of the water column integrated rates of BP (μg C m^{-2} d^{-1}) with a variety of estimates of sediment BP obtained from the literature, suggests the sediment rates at the maximum depth sampling sites to be no lower but more likely about an order of magnitude higher than the measured water column rates. If confirmed experimentally, the results point to an uncoupling of water column and sediment production, at least in our primarily oligotrophic forest lakes, and secondly, the incompleteness, from a whole-lake perspective, of studying only planktonic processes when bacterial metabolism in all but exceptionally deep sites appear to be overwhelmingly benthic.

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Biodiversity: bridging the gap between condition and conservation

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Abstract

The aim of this study is to create a two-tiered assessment combining restoration and conservation, both needed for biodiversity management. The first tier of this approach assesses the condition of a site using a standard bioassessment method, AUSRIVAS, to determine whether significant loss of biodiversity has occurred because of human activity. The second tier assesses the conservation value of sites that were determined to be unimpacted in the first step against a reference database. This ensures maximum complementarity without having to set *a priori* target areas. Using the reference database, we assign site-specific and comparable coefficients for both restoration (Observed/Expected taxa with >50% probability of occurrence) and conservation values (O/E taxa with <50%, rare taxa). In a trial on 75 sites on rivers around Sydney, NSW, Australia we were able to identify three regions: (1) an area that may need restoration; (2) an area that had a high conservation value and; (3) a region that was identified as having significant biodiversity loss but with high potential to respond to rehabilitation and become a biodiversity hotspot. These examples highlight the use of the new framework as a comprehensive system for biodiversity assessment.

Introduction

The assessment and conservation of biodiversity has been one of the most important topics in both academia and natural resource management in recent years. Although the United Nations (UN) Convention on Biological Diversity (UNCED, 1992; UNEP, 1992) is widely recognized, it specifies neither trait, nor method of quantification of biodiversity (Zeide, 1997). The Convention, however, defines biodiversity as "the variability among living organisms from all sources (. . .) and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems". This implies that there should be different measurement and management tools for each scale (Bass et al., 1998; Lister, 1998).

The current debate on biodiversity can be divided into academic versus applied goals (Srivastava, 2002). The academic side of biodiversity research mainly focuses on the links between biodiversity and ecosystem function/stability, using process-based analysis to set conservation strategies (Bengtsson, 1998; Lister, 1998; Schwartz et al., 2000). Applied studies, geared towards managing biodiversity, take a more reductionist point of view (Lister, 1998), quantifying species and populations.

Applied biodiversity approaches can be separated into two major groups. Most UN efforts at present are focussed on status, trends and causes of biodiversity loss (UNEP, 2003). The most commonly used framework for these restoration efforts is the Pressure-State-Response framework, developed by the OECD and described in Cairns & Pratt (1995). Species loss has been discussed on local (Crist et al., 2000), national (Smith, 1996; Roper-Lindsay, 2000) and global (Hogg et al., 1996; Williams, 2000) scales. These studies are also the basis for ecological risk assessment systems (Freedman & Beauchamp, 1998; Kamppinen & Walls, 1999; Reyers & James, 1999), geared towards applied ecosystem management. This branch of applied biodiversity research is mainly focussed on areas with a high human influence that puts ecosystems at risk.

The second branch of applied biodiversity science includes conservation studies. Instead of discussing loss of biodiversity, publications by Freitag & Van Jaarsveld (1998), Margules et al. (2002) and Myers et al. (2000) focus on the conservation of sites of special significance, mainly in areas that are not affected by human activities. Many of these studies deal with the problem of how to identify and protect areas of high conservation value, based on species richness, and endemism or rarity (Mittermeier et al., 1998; Noss, 2000). While conservation studies are often confined to areas with low pressure from human activities (Pressey et al., 2000; Simonson et al., 2001; Desmet et al., 2002), Mace et al. (2000) call for a system that does not require *a priori* selection of target areas and that operates on a smaller scale than national and global assessments. In our view, a strategy that integrates the assessment of loss of biodiversity, combined with the selection of patches of special significance (or high biodiversity) would unify both branches of applied biodiversity research and create a powerful management tool.

Another extensively discussed topic is the technical aspects of biodiversity assessment. Although precision is always a pre-requisite for scientific studies, cost effectiveness is a key issue for diversity surveys (Danielsen et al., 2000; Gioia & Pigott, 2000). The concept of surrogates in biodiversity assessments has been widely accepted in the recent years. Surrogates are taxonomic groups that indicate the overall biodiversity at a surveyed site. Although multi-taxa studies are preferable, researchers recommend invertebrates as the group that will represent up to 90% of the genetic variation (Duelli, 1997). In biomonitoring surveys of aquatic systems, benthic invertebrates have played a key role for years, because they are ubiquitous and diverse (Rosenberg & Resh, 1993), relatively inexpensive to sample and many laboratories have a good working knowledge of their taxonomy (Hellawell, 1986). In this paper, we trial the use of benthic macroinvertebrates as surrogates for aquatic biodiversity in inland rivers.

The aim of this study is the development of a two-tiered approach for applied biodiversity studies. Our model system will be rivers in the Sydney water supply catchments, Australia; an area with patchy land use ranging from national parks and agricultural and urban systems. The first step will be the identification of areas with significant biodiversity loss using AUSRIVAS (Australian River Assessment System) (Simpson & Norris, 2000), a RIVPACS-style method (Wright et al., 1993) for assessing aquatic ecosystem health. After sieving out river reaches that suffered from significant biodiversity loss, we will identify areas of special conservation interest using the AUSRIVAS reference database, to determine a site-specific index of conservation value (areas with higher than expected richness). This two-tiered approach will merge both branches of applied biodiversity assessment and thus meet an important need for managing both condition and conservation.

Methods

Study area and sampling methods

The catchments that supply water to Sydney cover about 16 000 km^2 in south-east New South Wales, Australia. The major land-use types are:

- protected areas (native vegetation, forested mountain areas, national parks, nature reserves): 49%
- agricultural/forested sites (mainly sheep and cattle grazing): 49.5%
- urban areas: 1.5%

Thirty nine sites little affected by human activities (mostly in protected areas) were chosen for building a reference database. Some sites were also chosen from catchments adjacent to those used for water supply. Test sites chosen for assessment included 11 from protected areas, 15 in agricultural areas and 10 urban.

Macroinvertebrates were collected from edge habitats (slow flowing, with structure provided by aquatic or overhanging vegetation, tree roots, large woody debris or bank undercutting), using a kick-net 350 mm wide with 500 μm mesh for a total transect of 10 m, as described in Turak et al. (1999). A composite sample from each habitat at a site in proportion to its representation, analogous to Wright et al. (1993), was collected for the test sites to determine whether the edge samples were appropriate surrogates for the biodiversity of the site. Invertebrates were picked from the whole samples using a modified New South Wales EPA (Environmental Protection Agency) live-pick method (Turak et al., 1999) with a minimum of 200 animals retrieved. To ensure a rapid assessment, macroinvertebrates were only identified to family, apart from the orders Plecoptera, Odonata, Ephemeroptera and Trichoptera, which were identified to species.

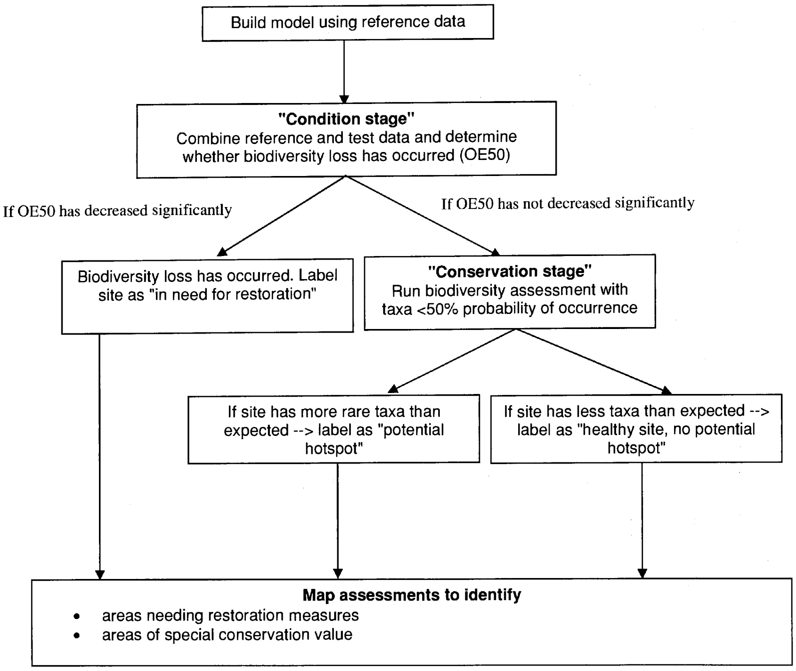

Figure 1. Flowchart for a two-tiered approach, integrating assessment of condition and conservation value.

Table 1. Coefficients for biodiversity loss (OE50) using taxa with a probability of occurrence >50%

	Observed (presence)	Expected (probability)	
Taxon 1	1	0.5	
Taxon 2	1	0.7	
Taxon 3	1	0.6	
Taxon 4		0.8	
Sum	3	2.9	O/E = 1.03

Assessment of condition and biodiversity loss

Site condition and possible loss of biodiversity (Tier 1, Fig. 1) were assessed using AUSRIVAS, the standard method for river health assessment in Australia (Simpson & Norris, 2000). AUSRIVAS predicts the probability of taxa occurring at a test site from a reference database of undisturbed sites (Turak et al., 1999; Simpson & Norris, 2000) by matching the environmental characteristics. Only taxa with a probability of occurrence higher than 50% are considered in the final assessment. By summing both probabilities of occurrence and the number of taxa collected, the coefficient of Observed/Expected (OE50) can be calculated (Table 1). The OE50 is a site-specific coefficient measuring loss of taxa. Only the more common taxa are considered (>50% probability of occurrence) and this makes the predictive model less prone to error by focussing only on the taxa "that should be there" to assess whether the normal state is present or biodiversity has been lost. To ensure a Type I error of 10%, the 10th percentile of the distribution of reference sites will be used as the cut-off for a significant loss of biodiversity. For example, if the cut-off is 0.8 and only 7 out of 10 expected taxa are found at the test site, a significant loss of biodiversity is detected. If a significant loss is detected, the site will not be considered for the second stage, the assessment of conservation value (see Fig. 1).

Assessment of conservation value

Conservation value is focussed on the rare taxa (Tier 2, Fig. 1) in contrast to the assessment of condition, which is based around the common taxa at a site

Table 2. Coefficients for conservation value OE(BIODIV) using taxa with a probability of occurrence <50%

	Observed (presence)	Expected (probability)	
Taxon 5	1	0.01	
Taxon 6		0.3	
Taxon 7		0.2	
Taxon 8		0.1	
Taxon 9	1	0.2	
Taxon 10		0.4	
Taxon 11		0.3	
Taxon 12	1	0.25	
Sum	3	2.46	O/E = 1.21

(defined by the probability of occurrence). Although 'rare species' is a high profile term in biodiversity literature, there are few working definitions or scientific criteria for making a determination. In this study we define a taxon that has a <50% chance of occurrence at a site as rare (in future studies, this could be shifted to a lower boundary). When using a reference database, this site-specific assessment of regional rareness, will define 'rare taxa' based on the operational management unit, which fulfils the requirements by Mace et al. (2000). The conservation coefficient OE(BIODIV) is calculated analogous to the OE50, but uses only taxa with <50% probability of occurring at a site (Table 2). While only 20–30 taxa might be included in the OE50, the whole remaining taxa in the reference database will be included. If the 250, or so remaining taxa in the database have an average probability of occurrence of 0.08, the expected number of rare taxa will be 250 × 0.08 = 20. If 20 taxa were found at this site, the site would be 'as expected', with an OE(BIODIV) of 1. If the observed number was greater than the expected, for example when taxa are found that do not exist in the reference collection or that are not expected in the particular area, the coefficient increases, labelling the site as taxonomically richer than expected or a possible conservation 'hotspot'.

Spatial analysis

To identify areas where restoration is needed or parts of the catchment that are of high conservation value, we mapped both condition and conservation assessment using ARCView 3.2 (ESRI, 1998).

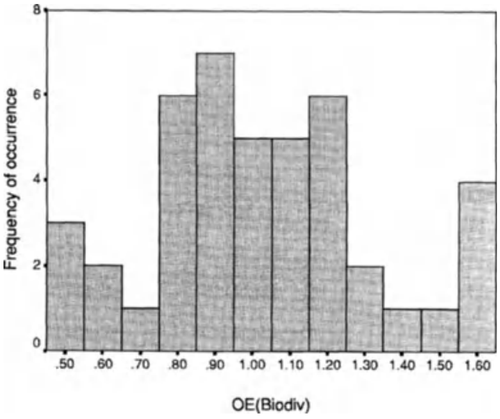

Figure 2. Histogram of OE(BIODIV) values for unimpacted sites in catchments of the Sydney region.

Results

The edge habitat can be seen as a good surrogate for the entire macroinvertebrate diversity at a site. The r^2 between species richness in the edge habitat and composite from all habitats was 0.5 ($p < 0.001$). At 1.3 species/family at sites on average, the number of species was correlated to the number of families at r^2=0.83 ($p < 0.001$), clearly indicating that effective assessment could be achieved with identifications to only family level.

The species level AUSRIVAS model developed using the 39 reference sites was acceptable. The correlation of observed to expected taxa in the reference sites (ideally 1) was r^2=0.48. The 10th percentile cutoff of OE50 scores to determine significant loss of biodiversity was 0.7

Of the 75 assessed sites, 29 sites failed the condition assessment (OE50<0.7), including 4 reference sites that necessarily failed by definition. Only one of the sites with urban influence and only three agricultural sites passed the assessment. Seven of the sites in near natural condition had loss of biodiversity. The conservation assessment was run on the remaining 44 sites and their OE(BIODIV) scores calculated (Fig. 2). The distribution is centred around 1 and quite wide (0.5–1.6, Fig. 2).

Mapping the scores revealed that the Clyde river in the south-west of the study area (Fig. 3) had several adjacent sites with high OE(BIODIV) scores, indicating many rare taxa and a high potential conservation value. Hardly any sites in the mostly agricultural

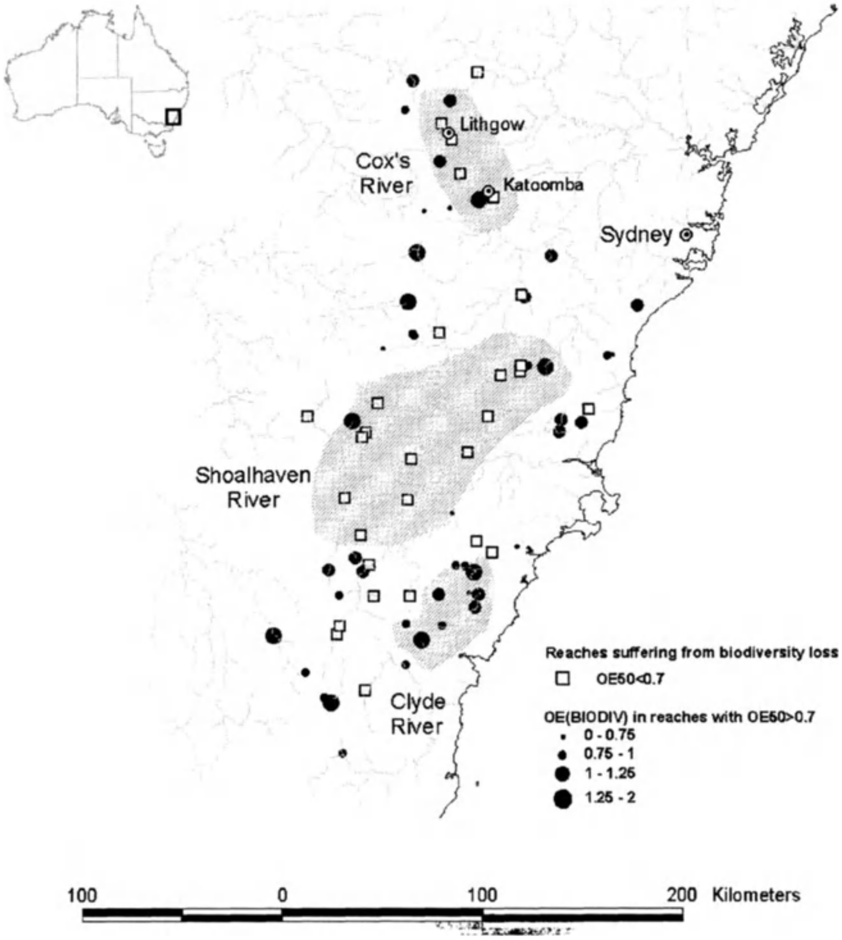

Figure 3. Biodiversity condition and conservation potential for rivers in catchments of the Sydney region.

central Shoalhaven valley passed the condition assessment. This highlights a significant loss of biodiversity and the need for restoration measures. The upper Cox's River in the Blue Mountains has many sites affected by human activities on the main stem, but high OE(BIODIV) values indicate that this catchment could be a potential biodiversity hotspot if provided adequate protection (Fig. 3).

Discussion

Our study demonstrates a potential method for addressing some frequently debated issues in applied biodiversity research. First, we attempted to integrate an assessment of biodiversity loss and the evaluation of conservation value. Second, we introduce a site-specific and comparable scoring algorithm to set conservation priorities, enabling a comparative evaluation at the scale of a management unit. Third, our study also tested the validity of rapid assessment using family rather than species level taxonomy and a single habitat rather than composite habitats.

Although we used mostly species level data for further analysis, the high correlation of family *versus* species richness and the low ratio of species/family at a site (1.25) suggests that family data would be an appropriate surrogate in future studies. Despite concerns about the use of family data in stream ecology (Lenat & Resh, 2001), there is evidence for the validity of lower taxonomic resolutions, both on an empirical (Marchant, 1990; Bailey et al., 2001) and a functional level (Thompson & Townsend, 2000). Error that may be introduced by using families rather than species may be more than made up for by using saved resources to sample additional sites that would greatly strengthen spatial assessment (Fig. 3). We are currently designing a future study that includes aquatic plants, diatoms and fish to test whether macroinvertebrates are an appropriate surrogate for biodiversity as suggested by Duelli (1997) for terrestrial habitats.

Another cost-reducing factor would be the concept of a 'representative habitat'. The high correlation of taxonomic richness in edge and composite habitat in the test dataset indicates that one habitat is sufficient to characterise the macroinvertebrate diversity in a study site. If it proves representative in further studies, a protocol using limited taxonomic groups, a relatively low taxonomic resolution (i.e. family or genus level) and a representative habitat would lead to a cost-effective rapid biodiversity assessment, analogous to the existing rapid bioassessments in the river health literature (Plafkin et al., 1990; Barbour et al., 1992). A rapid biodiversity assessment covering both loss and conservation potential would satisfy calls for cost-effectiveness (Danielsen et al., 2000; Gioia & Pigott, 2000) and would enable higher spatial resolution of the surveys meeting management needs on both counts.

The use of a reference condition approach (Reynoldson et al., 1997) introduces the ability to making objective comparisons in biodiversity assessments. The common reserve selection algorithms (Freitag et al., 1997; Margules et al., 2002) all include rarity of taxa as a value, an issue also discussed by Noss (2000) and Sarkar et al. (2002). A predictive approach from a reference database adds an objective, *a priori* defined measure of rarity within the study region, which gives more weight to unusual or endemic taxa. OE(BIODIV) (Table 2) is a site-specific measure of the richness of taxa that are rare within the study region. The approach is more than a mere stocktake of taxa richness. It compares the observed occurrence of rare taxa to the potential biodiversity of the site given its location and characteristics.

As a selection algorithm, the calculation of OE(BIODIV) is an attempt to resolve the problem of 'complementarity' (Faith & Walker, 2002; Sarkar et al., 2002). The principle of 'complementarity' in conservation theory looks for sites that add as many under-represented surrogates (taxa in this case) as possible (Pressey et al., 1993; Faith & Walker, 2002; Sarkar et al., 2002). Using a reference database that mainly contains protected sites as the benchmark will ensure an assessment based on complementarity. Sampling of taxa that are not ubiquitous in the reference database or not expected in a certain sub-catchment will increase the OE(BIODIV) and therefore the complementarity value of the site. This ensures the identification of surrogates in a data-driven and repeatable way (Desmet et al., 2002).

Following the global prioritisation of biodiversity hotspots (Myers et al., 2000), Mace et al. (2000) called for techniques in biodiversity assessment that would be applicable to a range of spatial scales, especially smaller scales that would have more relevance to management units. A reference-based approach would be applicable to all scales, yet might primarily be applied within management units. Reference databases are usually compiled by the same authorities that are responsible for future conservation and restoration planning.

For river bioassessment, reference databases have been established in many parts of the world. Apart from RIVPACS/AUSRIVAS predictive model approaches in Australia (Simpson & Norris, 2000) Great Britain (Moss et al., 1987), Canada (Reynoldson et al. 1997), Spain (Alba-Tercedor & Pujante, 2000) and Indonesia (Sudaryanti et al., 2001), other approaches using reference data have been applied in the Yukon territory (Bailey et al., 1998) as well as highly urbanized areas of Canada (Linke et al., 1999) and Europe (Wimmer et al., 2000). Reference databases also exist in most areas of the U.S.A., either used for RIVPACS models (Hawkins et al., 2000) or multimetric assessment (Karr & Chu, 1999). These databases could be readily used or enhanced. Depending on the spatial scale and resolution, as well as the taxonomic level, new databases can be relatively inexpensive to build.

Apart from adding innovative approaches to conservation planning, the main aim of this study was to integrate the assessment of condition and conservation value (Fig. 1). The two-tiered approach proposed in this contribution satisfies the criteria specified by Mace et al. (2000). In their critique of Myers et al. (2000) they called for a system that has no *a priori* selection of target areas for biodiversity studies.

Confining conservation efforts to natural areas discards the information of areas with mixed land use and does not maximise complementarity. The case studies in the three sub-catchments used in our study illustrate the flow of a possible management system. The Clyde catchment in the south-east of Figure 3 is an area largely in reference condition. Almost all of the sites score above average in the OE(BIODIV), some as high as 1.6, indicating that the macroinvertebrate composition at these sites is unique and has high conservation value. The condition of the middle Shoalhaven catchment suggests that this area suffered from a severe loss of biodiversity, highlighting the need for restoration measures. At this stage, an assessment of the conservation value of the middle Shoalhaven catchment cannot be provided, because almost all of the sites were assessed as suffering biodiversity loss. The upper Cox's River catchment demonstrates the real value of a two-tiered approach: Although the sites on the main stem, downstream from the urban centres of Lithgow and Katoomba have suffered a loss of species richness, the unimpacted tributaries have a high complementarity value. This suggests that with appropriate catchment management, the upper Cox's River could be restored to be a major hotspot for macroinvertebrate diversity and worthy of conservation. The area is taxonomically rich and can readily provide colonists to rehabilitated areas also indicating that it is likely to be responsive to management intervention. This conclusion could not have been reached with a traditional approach that only targets untouched areas for conservation management and highlights the need to integrate condition and conservation.

This study was a pilot project, intended to demonstrate the philosophy of the approach. Issues of taxonomic and spatial resolution, as well as adequate surrogacy of macroinvertebrates will be examined in a follow-up project. It also seems desirable to include socio-economic factors into the decision tree as demonstrated in Faith & Walker (2002). Overall, the reference condition guided, two-tiered approach, addresses many issues raised in the recent literature. It is cost-effective (Danielsen et al., 2000; Gioia & Pigott, 2000), data-driven and repeatable (Desmet et al., 2002) and adds a quantitative, comparative approach to biodiversity assessment (Duelli, 1997). Integrating condition and conservation avoids the problem of information loss by setting *a priori* target areas (Mace et al. (2000)) and maximises complementarity. Simple outputs that can be applied at large spatial scales will aid both restoration decisions and identification of conservation priorities and thus will provide a more comprehensive tool for biodiversity assessment that meets an urgent need for managers.

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Cultural associations in an ancient lake: gods of water in Lake Biwa and the River Yodo basin, Japan

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Introduction

Lake Biwa is the largest lake in the Japanese Archipelago (surface area 670.3 km^2; volume 27.62 km^3). An ancient lake, it originated about 4 MY-ago, and has become deeper and larger at the present site during the past 0.4 MY-ago. It was never glaciated. Some endemic species of fish, e.g. *Silurus biwaensis*, pre-date 0.4 MY; many others have evolved in the lake, such as *Gnathopogon caerulescens, Sarcocheilichthys biwaensis, Carassius buerugeri granoculis, C. cuvieri, Oncorhyuncus masou* subsp. and even the Lake Biwa form of *Plecoglossus altivelis*. Human beings have lived around the lake for over 20 000 years, and more than 200 ruins have been found on the lake bottom. The most comprehensive evidence of relationships between people and the lake is Awadu Lake Bottom Ruins, the largest shell mound in the world. Written records on human/lake relationships have been kept since the 8th century. So, Lake Biwa may be called an ancient lake from cultural as well as physical and biological aspects (Kawanabe, H., 1999).

After 794 AD, the capital of Japan was in Kyoto as 'Heian-kyou', located between River Kamo-gawa (east), River Katura-gawa (west) and River Uzi-gawa (south), flowing from Lake Biwa, which join each other and become River Yodo-gawa running into Osaka Bay at the east end of Seto Inland Sea. Since that time, Lake Biwa and the River Yodo-gawa have been used as main routes of transportation between Kyoto and districts in the Japanese Archipelago, and even to and from the Korean Peninsula and Chinese Continent. Many and various types of gods and sacred sites associated with water in the area have been places of prayer since that time.

Gods of water in or around Lake Biwa

Around Lake Biwa, there are many evidences of the great influence of Korean culture. For example, the Seta Bridge, which spans the mouth of the outflow of Lake Biwa, in the 7th century had a strong likeness to Korean ones, particularly the base of the bridge. Many shrines, temples and burial mounds are of Korean origin. The most famous god and sacred site around Lake Biwa, however, is dedicated to the Goddess Benzai-ten on the Island Tikubu-sima.

Tikubu-sima and the Goddess Benzai-ten

Tikubu-sima is the second largest island in Lake Biwa (14 ha). It is rocky and located in the northern part of the lake. Water depth around the island is about 60 m. It is one of the most sacred places in Japan (e.g., Hayasaki, 1978).

Since ancient times, human beings lived around the lake or travelled across it to pray at the island as a sacred site. A Buddhist temple, Hougon-zi, was settled on the island in the late 8th century. Later a Shinto Shrine, Tukubusuma-zinzya, one of the registered shrines in 'Engi-siki', was established there (Hudihara et al., 927).

The Buddhist temple is sacred both to Kanzeon-bosatu (abbrev. Kannon), Avalokitsvara in Sanskrit, the Saint of Mercy, and to Benzai-ten (abbrev. Benten), Sarasvati, which, in Sanskrit means 'one having water', was originally a Hindu goddess but later became one of the angels serving Buddhism and characterised by cleverness, conversation and music. The latter was believed in Japan to be a god of property, and has become the only goddess of the Seven Deities of Good Luck. Her favorite musical instrument is the biwa, a four-stringed lute. It is thought the lake may have been named Lake Biwa from the fact that its shape is similar to the lute. More precisely, however, the origin comes from the lute of Benzai-ten, who is a mixture of many gods and has been widely and devoutly believed in by Japanese for long time (Kimura, 2001).

It is also believed that the goddess Asai-hime-no-mikoto fought with the god Tatami-hiko-no-mikoto in ancient times and came to the island, where she became the principal image of the shrine in the 10th century. From the 9th century, Buddhism and Shintoism were mixed with each other. Thus, the Buddhas and gods of Buddhism have been repeatedly born as human beings or other gods, and they also came to Japan with the names of gods of Shintoism. For example, Amaterasu-Oomikaki, the ancestral goddess of the emperor family, was believed in Japan to be the incarnation of a Buddha of the Great Sun, Dainiti-nyorai. In the same way, the goddess Asai-hime-no-mikoto was believed to be derived from the angel Benzai-ten. These beliefs continued until the later half of the 19th century, when Buddhism and Shintoism were decreed by the modern Japanese Government to be distinct and separate.

The catfish, messengers of Benzai-ten in Tikubusima Island

As well as in Tikubu-sima, Benzai-ten is famous in the islands of Eno-sima near Kamakura and Ituku-sima near Hirosima. There are many other shrines or temples sacred to the goddess in the Japanese Archipelago. Usually, a messenger or servant animal for her is represented by a snake or dragon or some mixture of both. In the case of Tikubu-sima, the messenger of Benzai-ten is a dragon and also a catfish.

Hitomi (1692) wrote that "A marine fisherman came to Kaidu, at the northern shore of Lake Biwa, and dived to the bottom near the Island Tikubu-sima. More than 4 hours later he returned and reported that in big holes under the island he found not dragons but many catfish, enormous in size and impossible to measure". Terasima (1715) wrote that "at moon nights of mid-autumn, hundreds and thousands of catfish jump on the northern sand bottom of the island, because Goddess Benzai-ten loves them". There are three species of catfish in the lake, two of which are endemic. All come to shore and even enter rice-fields around the lake for spawning in early summer. Such behaviour is not observed in autumn, however.

Gods of water in and around Kyoto City

Kibune Shrine as a typical god of riverheadwaters

The most famous shrine or temple associated with water around Kyoto is probably the Shrine Kibune-zinzya, 15 km north of Kyoto City along River Kibune-gawa, one of tributaries of River Kamo-gawa. Since establishment of 'Heian-kyou', a god of water 'Takaokami-no-kami' has been revered for rain and flood control, and the shrine was ranked high from 818. It is one of the registered shrines in 'Engi-siki' completed in the 10th century (Hudihara et al. 927), and on every occasion necessary to pray for rain, the government has sent senior officials to the shrine (Hudihara Syunzei in Hudihara, R., 1192). The shrine was called just as 'The God of River-head' (Kamo, Y. in Minato, M. et al., 1205).

Later, the Shrine of Kibune-zinzya was recognized by the general public as a god for matters of love as well as of water (Idumi-sikibu in Hudihara, M., 1086; Anon., 1480s).

Hatiman-guu Shrine as a typical god of lower river waters

The other famous shrine or temple associated with water around Kyoto is probably the Shrine Iwasimidu-Hatiman-guu, 15 km south of Kyoto City at a hill near the juncture of River Kamo-gawa, Katura-gawa, River Udi-gawa and River Kidu-gawa, or starting point of River Yodo-gawa. Before the establishment of 'Heian-kyou' a Buddist temple had been there, and in 859 the gods 'Hatiman-Daibosatu', 'Hime-no-Oomikami' et al., which has been revered for water, sea, slash-and-burn farming and smithery (Zouki-housi in Hudihara, M., 1086; Hudihara, Y., 1228), was invited officially from Usa Shrine in.Kyusyu.

Later, the Shrine of Hatiman-guu was recognized by the court and knights of Shogunate as a god of war and military as well as of water.

Wells in and around Kyoto

Inoue (1933) collected and described 200 wells, smaller and more private sacred sites in and near Kyoto. Almost all the wells have their own god, but the relations of a well to its god are not the same in each case: 30% of them are believed to have been made by a god or a great person, and in 50% of cases the well itself is the god. In the case of stones or rocks, on the other hand, 60% of them have a legend that a god came, and for 20% the stone itself is the god.

Examining Inoue's description, historical trends appear quite different in the two cases. In the case of stones, a god appeared to monks or the public, and then the rock itself became a god and was given the name of the particular god. In wells, on the other hand, a well itself had been sacred as god and later a famous god came there for praying, celebrating or using the well, making the site sacred to that god.

Relation between gods in Japanese waters

The gods of water

In ancient days in Japan, the gods of water were called Mituha-no-me-no-kami, Kura-mituha, Taka-okami, Kura-okami, Mikumari-no-kami, etc. They lived everywhere in land and water and governed all things related to water, such as rain, spring, river, lake, wetland, well, cave and groundwater. In some cases, however, they had different and separate professions, such as handling drinking water, fishing and fisheries, transportation, flood control, and rescue from drowning.

As well as a dragon or snake, water imps, 'Kappa', are recognized as messengers of a god of water, especially in the western part of the Japanese Archipelago. Kappa are amphibious and in size similar to a child of 4 – 5 years old. Festivals occur usually in early summer; and people pray the gods for water to supply rice fields, for control of insect pest and plagues, and to exorcise evil spirits, etc.

The gods and goddess of water are acknowledged everywhere. Small flower decorations with or without other offering are seen even now in villages, at river sides and in rice-fields . The headwaters of streams, however, are given distinguished status as sacred sites, and as was shown above Shrine Kibune is the typical example of the category. 'Kawa-suso' Festival is also very important and usually held at the junctions of rivers or at river mouths flowing into the sea. They take place especially in Kinki District and mainly in the second half of June. Shrine Hatiman-guu mentioned above is its typical case. The cleaning of rivers and repair of installations in water are carried out by the people of the villages and towns.

The god of rice-fields

Rice-fields comprise important bodies of water in Japan. So, of course, there are many associated gods and goddesses. In ancient times the festivals were called Ukatu-no-mitama, Ukemoti-no-kami, etc., and these occasions are still held at present as festivals in the court: i.e., at Kanname-sai (17 Oct.) and Niiname-sai (23 Nov.) For the public, however, such religious festivals now include other kinds of god, e.g., I-no-kami, Ebisu, Daikoku, Inari, Zi-gami, Nou-gami, Saku-gami, Tukuri-gami, Kamado-gami, Kou-zin, O-syaniti-sama, O-usi-sama.

Festivals are held before seed planting (usually 15 January in lunar calendar), at the beginning and end of planting, at beginning and end of harvest, and after threshing (October in lunar calendar). Inoue (1933) noticed that flower decorations, sometimes with the display of a talisman, were made at the inflows of water to rice-fields before planting the rice-seedling all around Kyoto city. Flowers of azalea, camellia, chestnut, globet lower, peach, and/or branches of bamboo, thatch and sakaki (sacred wood, *Cleyera ochnacea*) are usually used for this purpose. After planting the seedling, the decorations are thrown into the river and children go round the village to beg offerings to the gods which are eaten in the evening. Symbols of sex are sometimes used for inviting the gods to the rice-fields.

In some parts of Japan, the gods of rice-fields were believed to be the children of the gods of mountains. In many villages, people believed, or were supposed to believe, that the mountain gods came to the rice fields in spring and went back to the mountains in autumn. Main festivals occur in the two seasons when the gods pass between the mountains and rice-fields.

Postscript: religions as functional relationships with nature

Water itself has been sacred to the Japanese people from ancient times. Spring, stream, lake, river, wetland, underground water, etc. were believed to be the god itself rather than simply symbolic. After the World War II, especially during recent 40 years, however, life-style of most Japanese has been greatly changed including manner of use and waste of water, and traditional religion and sacred sites have been partly destroyed.

In the 13th century, a Buddhist priest Sinran (1258) wrote as follows: "Nature was not made by any outer forces but was made of its own accord. Buddha or all religious absolutes are means of understanding the state of nature. After real understanding is reached of nature and Buddha, it should not be open to discussion. If it becomes a matter of discussion, then nature would not be made naturally and by its own accord. This understanding is the miracle of Buddhism".

Sacred sites, especially the ones mentioned related to water, are not now generally regarded as having a god present. Instead we recognise the sites as symbols of nature as it has evolved and is manifested by biological communities and their relationships with their environments. In the same sense we can see a symbolism between the sites and human cultural diversity, which essentially is related to biological and environmental diversities.

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Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries

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Abstract

One of the most insidious threats to fish conservation around the world is deliberate or accidental introduction of fish species. The impact of alien invasive sport fish is for the most part unpredictable in time and space, with the introduction of relatively few species having resulted in many extirpations of indigenous fish species worldwide. More nations need to quantify biodiversity loss caused by alien sport fishes. The spread of alien invasive fishes does not respect political boundaries. Therefore total global costs to aquatic biodiversity and ecosystem functioning resulting from these introductions need to be assessed. The global invasive species database of the Global Invasive Species Programme, highlights eight fish species among the one hundred 'World's Worst Invasive Alien Species'. Three of these fish species (two trout and one bass species) were introduced solely for sport. Historically the social value of recreational fishing was usually more important than conserving biodiversity. Globalisation of alien fish species for sport is best illustrated by rainbow trout – now in 82 countries, and still spreading, along with the associated expensive angling gear, magazines and accommodation infrastructure. Such sport species have become part of the global consumer society. The nature and extent of the globalisation phenomenon is addressed with regard to how introduction of alien fish for recreational angling has impacted on biodiversity; trophic cascades at a local level and the unassessed total cumulative global trophic cascades; and some of the motives that underlie promotion of this sport within the complexity of globalisation as we know it today. Alien invasive recreational fish species are now recognised as a global environmental degradation problem resulting in loss of biodiversity and therefore require a global solution. Parallel trends such as globalisation of environmental education and the internet must be encouraged to counteract the damage caused and reverse the trend. This globally concerted campaign requires utilizing environmental education forums aimed at the angling community, general public and policy makers; networking with existing alien invasive groups; legislation; better understanding of processes; development of environmental economic evaluation tools; international bio-invasion control; wider use of the precautionary approach and utilization of the present globalisation of ecological thought.

Introduction

Anthropogenic perturbations to freshwater systems over the past 100 years have escalated due to burgeoning human populations. Estimates of the number of freshwater fish species that will become extinct within the next 20–30 years run as high as 3000 species, about 30% of the 10 000 known species (Stiassny, 1998). This is why conservation of freshwater fish species is seen as a priority throughout the world.

Freshwater fish are threatened by habitat degradation and fragmentation; species introductions and translocations; impoundment of rivers (dams and weirs, water abstraction and water transfer schemes); and water quality deterioration and overexploitation (Cowx & Collares-Pereira, 2002).

At a UN conference on alien species in Norway in 1996 experts from 80 countries concluded that alien invasive species were a major threat to biodiversity conservation and probably the greatest threat after habitat destruction (Neville & Murphy, 2001). This holds true for freshwater fish species (Cambray, 2000; Cambray & Pister, 2002). Invasive aliens are known to represent a major global change issue (Mooney, 1998) and this is clearly evident in freshwater fish diversity loss. Lowe-McConnell (1990) noted that one of the most insidious threats to fish conservation around the world is deliberate or accidental introduction of new fish species. Introduced species that reproduce successfully can have major cumulative effects over time (Orians, 1995). Benefits of reducing cumulative effects of the spread and impact of alien sport fish species are long-term and societal. One third of all endangered and threatened species in the U.S.A. are listed, at least in part, due to the action of alien species (Bright, 1995). Nineteen endemic fish species are associated with the Cape Floral Kingdom of which 15 are threatened with extinction, primarily due to impacts of invasive alien sport fish species and habitat degradation (Impson et al., 2002).

The international community recognises the need to protect biodiversity (e.g. IUCN Red lists) and biodiversity conservation has become politically important (Cowx & Collares-Pereira, 2002). But very few countries actively undertake their responsibilities under Article 8(h) of the Convention of Biological Diversity (CBD) (Neville & Murphy, 2001). The CBD calls on all contracting parties to prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats and species. Therefore where introductions have occurred alien invasive species need to be controlled and if possible eradicated.

Certain freshwater fish species used for recreational angling have been transported around the globe and placed in rivers, dams and lakes, frequently without environmental impact assessments or monitoring, for the sole purpose of providing 'enjoyment' for anglers. These introductions have resulted in subsequent loss of biodiversity in the receiving systems.

The introduction of alien species has dramatically changed many ecological communities and contributed to species extinctions, most notably freshwater fish (Orians, 1995). This movement of species by humans has led to a breakdown in biogeographic barriers (Elton, 1958), both within and between continents, which had previously been the reason for the distinctiveness of the world's biota.

Fish conservation requires robust, defensible, social and economic evaluations of fish populations and species diversity (Cowx, 2002a). So-called 'free services' that unperturbed aquatic biota offer must be factored in. Justification for conservation of small species, not attractive to the angling community, is difficult. Their use as forage fish for a large alien species must be discouraged. "To reverse these philosophies is going to be a major challenge to conservation managers, but will be achieved if the true economic value of conserving fish species is not enunciated or the fishing fraternity are not educated in deleterious effects of introductions and translocation of fish species" (Cowx & Collares-Pereira, 2002). Understanding the ecosystem processes involved is a way forward. Severe reduction of benthic invertebrates with resultant increase in periphyton is one way to argue for not stocking some alien species.

The angling public does not fully recognise the need to protect biodiversity therefore innovative approaches and global co-operation between countries and organisations are needed. Fish conservation officials face the problem of a multiple user environment. In many cases, indigenous fishes are considered of marginal importance (Cowx, 2002a). Alien species are better known due to their established economic value and global literature on the species compared to little known and often poorly studied indigenous species. Many members of the public, such as farmers and many freshwater anglers, still believe that we can improve on the initial biotic 'hand' (Mooney, 1998) that was dealt to any river system by translocating or importing alien sport fishes. W.R.Courtenay, Jr. (pers. comm., 1999), after dealing with alien fish problems for many years, wrote: "I never cease to be amazed that the human species in its migrations over time and particularly within the past and present century seems to feel that introductions make things 'better'. This has proven true, but for a limited number of introduced species such as crops and certain 'domesticated' livestock. Some of those, however, caused substantial damage to receiving ecosystems. The introduction frenzy went far beyond what should have happened and is now clearly proven to have been a mistake when humans introduced species for the sole purposes of 'enjoyment' (= sport, forage for sport fishes, and the aquarium hobby). Over many parts of this planet, those mistakes are now established as reproducing, often range-expanding species (plants and animals), more often than not destructive for some to most species of native organisms. Homogenizing of the biological resources of this planet is happening and will prove to be a major error, made from stupidity of the consequences. I've been in South Africa and have seen how introduced plant species have become dominant in many areas and sampled waters there where introduced fishes are more common than native species. I've seen the same in eastern Australia and, for nearly all my life here in the U.S. (and I'm now 65)."

Definitions

Alien species (non-native, non-indigenous, foreign, exotic) means species, subspecies, or lower taxon occurring outside of their natural range (past or present) and dispersal potential (i.e. outside the range they occupy naturally or could not occupy without direct or indirect introduction or care by humans) and includes any part, gamete or propagule of such species that might survive and subsequently reproduce (http://www.iucn.org/themes/ssc/pubs/policy/invasivesEng.htm#anchor392619).

Alien invasive species means an alien species which becomes established in natural or semi-natural ecosystems or habitat, is an agent of change, and threatens native biological diversity (http://www.iucn.org/themes/ssc/pubs/policy/invasivesEng.htm#anchor392619).

Alien transfers are any fishery practices that lead to modification of the natural composition of fish communities.

Ecocentrism is the knowledge that humanity exists within but as only one part of a larger life system.

Globalisation can be defined as "a social process in which the constraints of geography on social and cultural arrangements recede and in which people become increasingly aware that they are receding" (Waters, 1996 in Germain, 2000a), although there are critics of this definition (Germain, 2000b). The term entered our everyday vocabulary around 1960 (Waters, 1995).

Globalisation in the context of the present paper is the process of spreading various animals (e.g. alien fish) and experiences (e.g. angling for that species) to all corners of the earth. Sport fishing has an economic marketable component and is a large global industry.

Recreational angling can be defined as 'Fisheries conducted by individuals primarily for sport but with a possible secondary objective of capturing fish for domestic consumption but not for onward sale' (FAO, 1997). For the majority of anglers, fishing is a pastime for pleasure. For purposes of this paper Carlton's (1975) definition will be used: 'recreational fishing is the ritual pursuit of pleasure associated with experience.'

Zoogeographic pollution occurs when organisms are moved out of their known home range and introduced into new areas by humans. If the organisms are closely related to organisms in the new environment there can be loss of genetic integrity.

Discussion

History of recreational fishing

Angling, like hunting, had it origins as a means of obtaining food. Reference to recreational fishing date back to 1496 when angling as a sport was propounded in the 'Treatyse of Fysshynge wyth an Angle' by Dame Juliana Berners. The well known 'The Compleat Angler or Contemplative Man's Recreation' by Izaak Walton, published in 1653, set the scene for the importance of recreational fishing in the coming centuries. It was also probably the commencement of global homogenisation of freshwater angling species. Well known recreational fish species, such as rainbow trout, were introduced into 'virgin' waters as humans developed faster means of transport for themselves and their favourite angling species around the globe.

Some of the trends over time of translocating alien sport fish species are summarized in Table 1. In the 19th and early part of the 20th centuries, 'good intentions' fuelled the spread of alien recreational fish. Anthropocentrism, with the attendant drive to control nature, was clear in the early stockings of rainbow trout in many countries. Introductions were thought to 'improve the biodiversity' in local water bodies for anglers (Hey, 1926). Species were imported specifically to get rid of indigenous species, which may have 'interfered' with the success of introductions, such as trout (Hey, 1926). These 'well-intentioned introductions' have resulted in serious detrimental effects to natural ecosystems and in some cases extirpation of indigenous species resulting in trophic cascades (McDowall, 2003). In some National Parks in South Africa, the number of alien freshwater fish species exceeds the number of indigenous species (Russell, 1999, 2001).

With the intensification of globalisation in recent decades, movement of species around the world has become much more widespread and more difficult to control. Once introduced, alien sport fish species tend

Table 1. General trends in the history of the translocation of fish for recreational angling

	19th Century	20th Century first half	20th Century second half	21st Century
Public awareness level of importance of biodiversity	Ignorance	Ignorance	Increasing awareness	Greater awareness
Impact studies before translocation	None	None	Increasing	Increasing
Knowledge of indigenous aquatic biota	None	Increasing	Good	Good
Motivation for introduction	Acceptance of what was known in country of origin	Acceptance of what has already been introduced	Acceptance of what has already been introduced	Acceptance of what has already been introduced combined with, new trend - fish indigenous
Angling population pressure	Few dedicated anglers	Increasing number of anglers	Increasing number of anglers used to alien fish, see aliens now as 'indigenous' species	Possibly - decreasing number of anglers as in California
Legislation to protect aliens	Little	Enacted	Being removed	None
Legislation to protect indigenous species	Little	Little	Increasing	Good, especially threatened species
Illegal movement of aliens	Not illegal	Not illegal	Increase	Possible decrease
Awareness campaigns	None	None	Few	Increasing globally
Removal of aliens	None	None	Few	Increasing removals

to spread either by natural means, via anglers and farmers or via engineering feats such as interbasin transfers.

It is not only the ecological processes that need to be understood (McDowall, 2003). The human factor has to be taken into the equation, as it is the sole driving force behind these introductions. An angler can now travel round the world catching the *same species* of trout in over 82 countries! This is an extreme case of 'homogenizing the biological resources' of the planet.

It is unfortunate that movement of fish around the globe has created an expectation and a desire to continue this trend amongst many anglers regardless of the impact on indigenous aquatic biodiversity. Recreational angling in fresh waters is now big business. It has thus become a sociological problem. For some cases, such as rainbow trout, a certain status has been created for angling for this species (Cowx, 2002b). Many people have grown up fishing for alien species, which they now assume, are indigenous. An extreme case is that some anglers in South Africa want alien rainbow trout to be declared an 'honorary indigenous species' because it has been in the country for over 100 years (Hamman, 2002).

Motivation for recreational angling

Angling for most people is a pastime for pleasure. The main motivation for angling is to be able to relax in pleasant surroundings with like-minded angling friends; the number of fish caught in many cases is secondary (Steffens & Winkel, 1999). Recreational angling is therefore a complex issue involving not only the aquatic ecosystem but also what provides pleasure to humans. Angling for indigenous species can often fulfil all requirements noted above.

Driving forces

Certain driving forces promote alien fish for recreational angling.

Human migrations

As human populations colonised new territories and transport became more efficient it became easier to translocate fish species for recreational angling. This then began the process of zoogeographic pollution and homogenising what fish were acceptable to anglers on a global basis. In Italy, there are only 17 native species that have not been translocated to new localities or 'polluted' by introductions of conspecifics or alien fish species (Bianco, 1990). Major impacts have been the result of the introduction of transalpine species and translocations, river by river, of indigenous species that through movement become aliens in rivers into which they are introduced (Delmastro, 1986 in Bianco, 1990). The unique assemblage of freshwater fishes in Italy has been severely and irreversibly altered due to incorrect management practices. The result is zoogeographic pollution, loss of genetic identity of local populations, a high level of hybridisation and extinction or reduction of local communities of endemic species (Bianco, 1987 in Bianco, 1990). Most of this is the result of provincial authorities stocking public waters with a mixed bag of fish (Bianco & Ketmaier, 2001).

Angling promotion

Currently in 22 European countries there are at least 21.3 million anglers who spend large sums of money yearly on their sport (Cowx, 2002b). Marketing is a complicated network. Briefly, fishing magazines sell advertising space to sports stores and land owners whose attractive advertisements offer accommodation and 'good' fishing. In addition, magazines make fishing look enticing in articles specially written for a target group such as business executives, adventure seekers etc. Should the majority of these articles promote alien fish, then an angling population emerges who accept fishing for these fish as the *status quo*. Sport store outlets sell tackle, specifically designed to catch bass or trout. This globalisation of specialised angling equipment helps drive the spread of alien invasive fish species. Thus marketing is driving anglers' perceptions and expectations.

The type of fishing that an angler enjoys is often seen as reflecting their social status (Cowx, 2002b). Freshwater game fishing is mainly dominated by wealthier sectors of society. Game fish anglers mainly fly fish for species such as salmon and trout. It is mainly demands of this group of anglers that has resulted in rainbow trout being introduced into at least 82 countries (Welcomme, 1988).

Many angling clubs tend to focus on alien freshwater fish species. Some angling clubs are well organised and members pay to have waters stocked with their favourite angling species. Private syndicates are developed that purchase mountain catchment areas to create exclusive waters for alien trout angling. Weirs and dams may be erected for the trout thus completely altering the river system and in some cases flooding valuable wetlands.

Side-lined in this homogenisation process were many potentially excellent indigenous angling species which now face extinction or are extinct. In a tourism advertisement for New Zealand (= circa 2000), under the title 'Pure 100% New Zealand', an angler is shown catching an alien rainbow trout! A New Zealand Grayling (*Prototroctes oxyrhynchus*) being caught would have been 'Pure 100% New Zealand' but this species is now designated extinct, one of the probable causes being introduced salmonids (McDowall, 1996).

In South Africa indigenous species have only recently appeared in the flyfishing spotlight due to dedication of conservation officials (Impson, 2001). Yellowfish (*Labeobarbus* species) are proving to be popular and excellent angling species and now adorn the covers of local angling magazines. In retrospect, there was no need to introduce bass and trout into South Africa where they now compete and prey on the juveniles of the indigenous angling species, some of which are now endangered (Skelton, 1987).

However, this has created a demand for yellowfish and there are now requests for private hatcheries to rear these species (Dean Impson, pers. comm.). This would over time lead to many of the problems raised by Bianco (1987 in Bianco, 1990). Movement of fish for sport fishing has a bad track record. It will not get better without a concerted educational effort together with well-enforced legislation.

Therefore, collective agents (eg. anglers, sports shops, sport magazines, hatcheries, accommodation providers etc) are encouraging the continued globalisation of these species through modern marketing practices. Environmental activists are concerned with aquatic biodiversity conservation (Cambray, 2002, 2003) and fulfilling the mandate of the Convention on Biological Diversity. A competing dynamic now exists between these groups.

Hatcheries

Hatcheries are expensive ventures (ponds, pumps, vehicles, trailers, feeds, medicines, staff etc.). In some countries, such as South Africa, conservation departments historically set up hatcheries to rear alien species such as North American bass and trout species (Cambray & Pister, 2002). Millions of alien fish were reared and demand created among farmers and anglers. Once established with 'conservation' taxpayers money such hatcheries are difficult to close down.

Alien fish introductions had a cascading effect and are now out of control in some countries. In Italy, conservation officials stocked alien trout into mountain rivers of national parks (Bianco, 1995) instead of working towards the conservation of aquatic biodiversity.

In the U.S.A., hatcheries endevouring to improve sportfishing have not taken sufficient care to protect genetic integrity of stocks of trout and salmon species. Formerly genetically distinct stocks are now genetically contaminated (Behnke, 1988).

Private fish farms are sometimes authorized to provide fish for translocation. There are 200 of such hatcheries in northern Italy (Bianco, 1995). Such hatcheries are centres for selling and distributing aliens to anyone who has the money to pay for them.

Globalisation of recreational angling species

One of the major factors driving evolution of the 10 000 freshwater fish species was the isolation of many populations. Technological advancements make it feasible to fly fertilised trout eggs by aircraft from Colorado, on the North America continent, and hatch them in Lesotho on the African continent within several days. In Lesotho the alien trout will impact local biodiversity as soon as they start feeding, or possibly earlier, through imported diseases or parasites.

In Italy, the process of change in the composition of the freshwater fish fauna has been summarized as successively as 'padanization' (the result of transplantation of native species from north to central Italy); followed by 'danubization' (introduction of Danubian species throughout Italy); and now 'globalisation' (the establishment of Iberian, Albanian, Asian and North American elements) (Bianco & Ketmaier, 2001). Many of the fish were introduced by provincial authorities to enhance 'angling species diversity'.

Stock manipulation

For recreational fisheries, attempts are made to enhance diversity of target species for anglers or to provide a species an angler would be willing to pay money to angle for. Stocking is thus a widespread, but also greatly abused, management tool in inland recreational fisheries (Cowx, 1998). Stocking of natural waters can have several aims: improve recruitment; bias fish assemblage structure to favoured species or maintain productive species that would not naturally breed in the system (Cowx, 1994). Cowx (2002b) noted that such stockings should be carried out so that there is no impact on indigenous fish populations and I add here to other aquatic biota. Cowx notes that stocking to enhance fisheries is frequently carried out with 'no due regard for the environmental or ecological consequences.' Stocked alien fish such as trout and bass species can impact indigenous species through competition, predation, loss of genetic integrity or by spread of diseases and parasites (Cowx, 1994, 1998; Cowx & Godkin, 2000 and see McDowall, 2003 below).

Catering for angling diversity by introducing alien species became a common worldwide practice in the 1960s and 1970s (Welcomme, 1988). Problems associated with this enthusiasm to cater to anglers has only fairly recently led to legislation and restrictions being placed on this 'Wild West' approach to stocking aliens in industrialized countries. 'Developing countries' were frequently targeted by fisheries agencies from the developed world leading to inappropriate stockings. Conservation money in some provinces in South Africa was still channelled into alien fish production as late as 2002 (Cambray, 2003). Cowx (1998) noted, as did Hey (1977), that some introductions, such as rainbow trout and large mouth bass have been successful. To these two authors 'success' must mean that the introduction was economically successful and/or successful in the eyes of anglers. As for conservation of biodiversity, these introductions were bad conservation practices.

Assessing alien impacts

In many cases, it is difficult to assess, quantify or predict the impact of introducing a fish species (Fausch, 1988; Moyle & Light 1996; McDowall, 2003). Earlier studies on impacts of alien fish on indigenous fish were simplistic noting mainly competition and/or predation scenarios (McDowall, 2003). It is now apparent that there are more subtle interactions with altered animal behaviours that impact availability of resources shared with other species and also through feedback effects that influence interspecific interactions (Power et al. 1985; Wootton, 1994).

When stream invertebrates are reduced in abundance by an alien predator, their behaviour changes as they become more cryptic, leading to less periphyton grazing, which may depress production of benthic insects resulting in the benthos being less accessible to indigenous fish predators (McDowall, 2003). Introduced trout profoundly affected the structure and composition of faunal assemblages in Californian High Sierra lakes. Large and/or mobile, conspicuous taxa, including tadpoles, large-bodied microcrustacean zooplankton and many epibenthic or limnetic macroinvertebrates were rare or absent in lakes containing trout (Bradford et al., 1998).

An accurate assessment of the impact of alien sport fish species is only possible if an accurate assessment of pre-introduction ecological and socio-economic environment already existed (Bartley & Casal, 1998). A major problem exists because many introductions of aliens were carried out in the 19th century and even into the 21st century. No environmental impact studies were undertaken before or after most of these introductions making it impossible to assess potential or actual impacts.

In Italy, it is easier and cheaper, in the absence of strong laws, to introduce alien species than to conduct a study on the possible impact of the newcomer to the unit of destination (Bianco, 1995). This outdated practice may still hold for many countries. The main driving force is economics of the known alien sport fish regardless of the long-term damage the transfer may cause to the non-game indigenous species.

In New Zealand, with its abundant cool, swift-flowing rivers, introduced trout had a good 'fit' with existing habitats (Moyle & Light, 1996). This resulted in superb trout fishing (Spackman, 1892) and concern for indigenous species only began to emerge in the 1960s (McDowall, 1968) but the nature of the impact by the aliens was not fully understood (McDowall, 2003). It is now known that where alien trout densities are high there are cascading effects on stream ecosystems, such as reduced benthic invertebrates as well as behavioural changes that can result in proliferation of periphyton (Flecker & Townsend, 1994; McIntosh & Townsend, 1996). In a study on indigenous galaxiids Townsend (1996) found that the best predictor of presence of galaxiids was absence of trout in over 198 sites examined. Galaxiids only existed in 'fringe' upstream habitats to which trout were excluded by natural barriers. Galaxiids have decreased in New Zealand streams due to alien trout impact on the invertebrate production, resulting in benthic invertebrate behaviour change. Galaxiids cannot now access the best foraging areas which reduces the food available to them (McDowall, 2003). In Lesotho the same holds true for the endangered redfin minnow (*Pseudobarbus quathlambae*) (Skelton, 2000).

Global Invasives Strategy

Conservation biologists recognize the effects of alien invasives as complex with many social, ethical, and legal aspects in addition to biological and ecological dimensions (Mooney, 1998). Economic effects of removal of alien sport fish are complicated in that entire industries have been developed for these species. Areas that can be rehabilitated must be identified and prioritised and others areas possibly conceded to the industry based on the aliens.

There is unification of effort to counteract the alien invasive threat by organisations such as the Global Invasive Species Programme (GISP), coordinated by SCOPE (Scientific Committee on Problems of the Environment), in conjunction with IUCN (World Conservation Union), CAB International and UNEP (United Nations Environment Programme). Aims of the Global Invasive Species Programme (GISP) are to:

1. assemble the best information and approaches for prevention and management;
2. disseminate them in the form of databases, manuals and capacity-building training programs to governments and communities; and

3. lay the groundwork for new tools in science, information management, education, and policy that must be developed through collaborative international action.

The Invasive Species Specialist Group (ISSG) is part of the Species Survival Commission (SSC) of IUCN. ISSG is a global group of 146 scientific and policy experts on invasive species from 41 countries. ISSG provides advice on threats from invasives and control or eradication methods to IUCN members, conservation practitioners, and policy-makers. The group's activities focus primarily on invasive species that cause biodiversity loss (*http://www.issg.org/index.html).*

One hundred of the world's worst invasive alien species

The Global Invasive Species Database (*http://www.issg.org/*) states: 'It is very difficult to choose 100 invasive species, from around the world, which really are 'worse' than any others. Species and their interactions with ecosystems are very complex. Some species may have invaded only a restricted region, but have a huge probability of expanding, and causing further great damage (e.g. see *Boiga irregularis*: the brown tree snake). Other species may already be globally widespread, and causing cumulative but less visible damage. The one hundred species aim to collectively illustrate the range of impacts caused by biological invasion.'

An analysis undertaken for the present paper indicates that the species ranged from three microorganisms to 14 mammals. All eight fish species noted are freshwater species (Table 2). Of the 100 species, five were introduced for sport (two mammals and three fish species). Four other fish species are used for angling enjoyment, which means they can be spread by the angler pathway (Table 3). That such a relatively high percentage of these 100 worst invasive aliens are freshwater sport fish is cause for concern.

Table 2. Number of species introduced for sport in the list of the 'One Hundred of the World's Worst Invasive Alien Species'. (Analysed from the Global Invasive Species Database: *http://www.issg.org/database/species/*)

Group	Number	Number for sport
Micro-organisms	3	0
Fungus	5	0
Land plants	32	0
Land invertebrates	17	0
Mammals	14	2 (deer and fox)
Reptiles	2	0
Birds	3	0
Aquatic plants	5	0
Aquatic invertebrates	8	0
Amphibians	3	0
Fish (all freshwater)	8	3 mainly for sport but 4 others also are used for sport as well as food fish

Ecological integrity

Environmental issues have gained prominence on the contemporary security agenda (Scholte, 2000). There is now a spotlight on many environmental issues, maintained by civic groups, think tanks, official agencies, NGO's etc. There is a wider acceptance of the fragility of life on earth with associated feelings of insecurity for humanity.

Each of the major anthropogenic global environmental changes of contemporary history has presented threats to ecological integrity. Declining biological diversity might even take the earth to a species depletion threshold beyond which the entire biosphere would collapse (Scholte, 2000). On a small scale this is what is happening with introduction of alien angling species in some rivers in New Zealand. Introduction of alien recreational fish species is resulting in behavioural changes and trophic cascades that are not fully understood (McDowall, 2003).

Global consciousness has promoted greater ecological awareness. Global environmental issues have become a prime source of insecurity in the contemporary human condition (Scholte, 2000). This is most clearly seen in such issues as the oil crisis, genetically engineered foodstuffs, globally transmitted diseases etc. Potential exists for global governance on environmental matters, e.g. 1987 Montreal protocol on ozone reduction.

Codes of conduct for hatcheries and global angling equipment suppliers should include specific and enforceable environmental clauses. Ultimately the end consumer, the angler, needs to be educated to conserve biodiversity.

Ecocentrism

Ecocentrism (Eckersley, 1992) opposes anthropocentrism as humanity is seen to exist within, but as

Table 3. Analysis of the eight fish species listed in the 'One Hundred of the World's Worst Invasive Alien Species' list: (Analysed from the Global Invasive Species Database (*http://www.issg.org/database/species/*)

Species	Reason for introduction	Impact
Clarias batrachus	Aquaculture but also for **sport** fishing	Indigenous fish and other aquatic biota
Cyprinus carpio	Aquaculture but also for **sport** fishing	Reduces water quality and destroys aquatic vegetation by uprooting it
Gambusia affinis	Mosquito control, (indigenous species could also have done this!)	Harmful due to predaceous habits
Lates niloticus	Food and **sport**	Contributed to extinction of 200 fish species in Lake Victoria resulting in devastating environmental impacts (Barel et al., 1985)
Micropterus salmoides	**Sport**	Impact on indigenous fish, crayfish, amphibians and insects
Oncorhynchus mykiss	**Sport**	Displace indigenous species by competition and predation, also impact on aquatic invertebrates
Oreochromis mossambicus	Aquaculture also for **sport** fishing	Omnivorous eats almost anything from algae to insects
Salmo trutta	**Sport**	Severe impact on indigenous fish (especially other salmonids), amphibians, invertebrates through predation, displacement and food competition

only one part, of a larger life-system. Human desires, such as stocking alien invasive angling species, need to be renounced in favour of ecological health if there is a conflict, which there surely is. Scholte (2000) noted that many indigenous people have promoted notions of aboriginal knowledge where human beings are integrated within and subservient to a natural order. The so-called 'Gaia' notion regards the planet earth as a living creature to which humanity owes its responsibility (Lovelock, 1979). Globalisation has been one of the forces in promoting ecocentrist knowledge.

Global ecological changes have raised awareness of damages that anthropocentric rationalism can inflict (Scholte, 2000). Some global ecological changes have been due to movement of plants and other species. Climate change and rapid loss of biodiversity reinforce that humanity depends on ecological conditions. Policy makers opt for a rationalist response of sustainable development. This approach then perpetuates humanity's subordination of nature in its hope to find techno-scientific solutions to environmental problems.

Due to the past anthropocentric approach there can be no doubt that techno-scientific solutions are now needed to combat alien sport fish introductions that are destroying freshwater biodiversity around the globe.

Ways forward

Understanding processes

Behavioural and dietary interactions, between alien and indigenous species, must be understood (McDowall, 2003). These processes form a basis for informed conservation protocols to explain why the eradication of alien sport fishes is required.

Precautionary approach

When new species are considered for introduction there should be a proper protocol carried out by trained staff within an appropriate government organisation. Guidelines are available from the United Nations Food and Agricultural Organisation (FAO, 1996). The precautionary approach (FAO, 1996, 1997) must be followed when there is insufficient data on the impact a proposed alien fish introduction will have on the receiving system.

There are four options with regard to movement of species around the globe (Ruesink et al., 1995):

1. let everything in;
2. keep everything out;
3. experimentally test everything before entry (essential); and
4. utilize available information for a risk analysis decision (crucial).

Option 1 happened during the 19th and early part of the 20th century. Option 2 should be applied on a global basis as sport fish can be viewed as non-essential so the risk is not worth any further movement of these species. However, as Option 2 is often impractical (Ruesink et al., 1995), Option 4 is the most realistic and similar to the precautionary approach. The exercise will provide reasons why the fish should not be moved that reasonable people would accept.

A moratorium should be imposed on all sport fish translocations solely for angler enjoyment. Angling for indigenous fish species should be promoted instead.

Legislation and environmental education

An 'integrated alien fish management system' must be developed that includes control options, details of the biology of alien sport fish, impact of the aliens and the economic, social and ecological impacts of control efforts that would be required.

In those countries with limited resources the power of globalisation should assure that trained staff and funds are made available.

There should be total eradication of the aliens in rivers and lakes, but this is a very costly procedure and probably impractical for large systems. Barriers can be utilised to prevent the spread of alien fish (eg. golden trout in California; Cambray & Pister, 2002). Biocontrol methods need to be explored but with great caution to avoid local extinctions of indigenous species (Bright, 1995). Fish eradication programmes utilising piscicides could kill indigenous species. After an eradication campaign constant monitoring and vigilance is required to prevent illegal re-introductions.

A more effective international system to prevent bio-invasions is required. Hamdullah Zedan, executive secretary of the Convention on Biological Diversity, believes that a 'stronger system' is required to prevent entry in the first place. But where entry has already taken place more effective measures are needed to stop invasive alien species from establishing themselves and spreading. Where eradication is not feasible or cost-effective, more needs to be invested in containment and long-term control measures (http://www.iucn.org/wssd/pressbook/news/wssd/pressihtaug2802.htm).

Legislation restricting or prohibiting introduction and movement of alien recreational fish species is not sufficient in itself, although it can be a powerful tool if there is proper enforcement and significant fines and vehicle confiscations. Cases need to be well publicised. In addition to good legislation an educational awareness programme is required. At a UN conference on alien species held in Norway it emerged that very few of the 80 participating countries had sufficient information or capacity to address invasive alien problems (Neville & Murphy, 2001). It is time to address this need on a global basis.

The scientific community urgently needs to articulate knowledge to a broad array of people, especially decision makers (Orians, 1995). Along with globalisation of ecological thought (Mooney, 1998) there has been development of globalisation of environmental education (EE) to educate on environmental matters that are a cause for concern, such as loss of aquatic biodiversity. Scientists concerned about the spread of alien sport fish should promote the development of suitable EE conservation tools.

Mooney (1998) stressed the need for scientists to communicate their findings to non-scientists. Good communication to the public should be seen as part of a scientific portfolio. Some non-governmental funding bodies make communication at the popular level part of research contracts (eg WWF). Communication of findings to the public and policy makers is now generally viewed as part of one's career development. Good scientific review articles, such as on impacts of brown trout (McDowall, 2003), need to receive a wider audience. Facts need to be properly packaged and disseminated by scientists to promote public awareness. Websites, such as Science-in-Africa (http://www.scienceinafrica.co.za/), provide easy access for dissemination of research findings in a popular format.

"We need more scientists to become involved in the crucial task of making sure that the best science available is being utilized in public understanding of environmental issues, and in policy making" (Mooney, 1998: 125). If a scientist is funded solely to provide new information only published in scientific journals, with no public exposure, then we as scientists have failed. Programs to train mid-career scientists in communication skills, as initiated by the Ecological Society of America, need to be evaluated and encouraged.

There is concern that if one starts early in one's scientific career that public engagement will consume essential time that should have been spent developing one's scientific credentials (Burke & Lauenroth, 1997). Others (e.g. Mooney, 1998), as does the author, believe that a scientist needs to be aware of societal relevance of one's work and discipline and make appropriate contributions throughout one's career. In the case of freshwater fish, and many other species and their habitats, there is no time to waste.

Considering the enormity of ecological damage caused by invasives, as well as funding spent on trying to control their spread, it is surprising that more money is not spent on educating the public about this problem (Mooney, 1998). This investment in education at all levels could have an enormous economic payback by reducing further spread of aliens.

Even with heightened public awareness of the value of biodiversity this knowledge alone will not provide sufficient safeguards (Orian, 1995). Protecting biodiversity is a moral imperative, without which society would adhere to the dictum 'Preserve species when it is economically favourable to do so, but eliminate them when it is not' (Orian, 1995).

Responses to cumulative environmental effects, such as the spread of alien sport fishes, have a significant moral element. Leopold (1949) wrote "It is inconceivable to me that an ethical relation to land can exist without love, respect, and admiration for land, and a high regard for its value. By value, I of course mean something broader than mere economic value. I mean value in the philosophical sense". It is this ethical relation to aquatic biodiversity that needs to be incorporated into all people.

In South Africa, the Working-for-Water programme trains unemployed people to run a business based on removal of invasive alien plants (van Wilgen et al., 1998, 2001). This programme has made the public aware of environmental cost of these alien invaders. We now need a similar programme for alien sport fish species, not only in South Africa, but globally.

Environmental economic evaluation tools and 'free services'

A value needs to be articulated for the social and economic importance of freshwater fish biodiversity. There is an urgent need to adapt environmental economic evaluation tools (Cowx & Collares-Pereira, 2002). There are economic evaluation tools for alien sport fish and it is the economics that keeps this threat active and spreading. Information is available for indigenous commercial species but not for many of the 10 000 known freshwater fish species.

There is a need for more awareness of the value of goods and 'free services', which are provided by ecosystems (Orians, 1995). Good quality freshwater is a scarce commodity in many parts of the world. If introducing a fish species, such as brown trout into New Zealand streams, can virtually clean out the annual benthic invertebrate production, change behaviour of invertebrates and indigenous fish species resulting in a periphyton build-up (McDowall, 2003) then is it worth introducing this alien sport fish? What are the other ecological processes that could be upset by these introductions? This potential loss needs to be understood and costed into the full assessment of the impact of alien sport fish species.

Orians (1995) raised an interesting point regarding responsibility. If landowners want compensation from society for conserving a wetland on their farm then they should also pay the costs if they do not conserve it. That is, they should have to pay for flood control, water purification and species conservation, provided by the wetland before they filled it. Similarly if landowners introduce alien fish into rivers which flow through their land then they should be responsible to pay for species conservation, water purification and clean-up charges if the alien sport fish species move up or down stream into a nature conservation area.

Global network of fish conservationists

Cowx & Collares-Pereira (2002) recommended a network of fish conservationists who would report successes and failures in conservation management thereby assisting removal of aliens and rehabilitation of habitats. Cambray & Pister (2002) discussed problems and successes of several conservation campaigns and this is typical of the information that needs to be more easily accessible. Similarly, Cambray & Bianco

(1998) suggested a website for the conservation of freshwater fish species as part of a conservation 'tool kit'.

As part of the global campaign to combat the impact of alien fish on aquatic biodiversity an efficient, functional network of concerned scientists and members of the public would be beneficial for conservation of fish diversity on Earth. This could work through the IUCN, with a well structured and inclusive website referring readers to other workers in the world, to their published and unpublished works and success and failures of their campaigns.

The network could work with the existing 'Global Invasive Species Programme' (GISP) that aims to forge cooperation to address a borderless issue (Neville & Murphy, 2001). GISP focuses on aliens that disrupt ecosystem processes and thereby threaten biodiversity, health and economics (Neville & Murphy, 2001). Alien sport fish fulfil these requirements. The programme is a network of scientists, lawyers, environmentalists, policy makers, economists, resource managers and others working together on the global invasive alien problem. The mission of GISP is to enable governments and organisations to use the best practices available to manage invasive alien species (IAS) and to promote development of additional tools and strategies needed to improve global management of IAS. GISP strives to promote collaboration and partnerships with a holistic and multi-sectorial approach, exactly what is required to control and eradicate alien invasive sport fish species. GISP has strong support from SCOPE, IUCN, UNEP, GEF and other national and international bodies (Neville & Murphy, 2001).

Improvement of the scientific basis for decision making on invasive species issues centred on 11 components – including establishing the background and scientific and social basis of invasive alien species problems; current status of invasives; their ecology; human dimensions of the invasive species problem; and the relationship between invasive alien species and global change. GISP addresses identification of pathways of invasion, early warning systems, methods for prevention, early detection and management, risk assessment, legal and institutional frameworks, economics of invasive alien species and educational programmes (Neville & Murphy, 2001). Some of this work on vectors and pathways of invasion has already been carried out in South Africa (Richardson et al., 2003).

Globalisation of ecological thought

In a book by Mooney (1998) entitled 'Globalization of ecological thought' it is noted that ecological research was originally global. In the middle of the 20th century it became more locally focussed, whereas today it is again more global in nature. In the 1950's ecological studies focussed on natural systems and Mooney (1998) notes human-modified systems were mainly ignored and alien organisms were generally neglected. But today many studies focus on 'untangling of the responses of biotic change to natural cycles from that of human impacts' (Mooney, 1998). Focus of ecological studies is now of a larger dimension due to the relatively recent appreciation of global impacts of humans on biotic systems, and this is what Mooney (1998) refers to as the 'globalisation of ecology.' This aspect of globalisation will help to reduce movement of invasive aliens through providing a better understanding such as outlined by McDowall (2003) for brown trout in New Zealand. Globalisation of ecological thought will help counter the present consumer society approach to alien fish introductions.

Concluding remarks

Globalisation resulted in the spread of alien freshwater sport fishes in an unprecedented and unnatural way. Globalisation and homogenisation of species is proceeding at an ever-increasing pace and have assumed their own dynamics. Only a co-ordinated counter global awareness campaign can assert the importance of indigenous freshwater fish species in the eyes of the public and policy makers.

There is a distinct need for a global assessment on impact of the most widely distributed alien recreational fish species, such as rainbow trout, on aquatic biodiversity and ecosystem functioning.

Globalisation power must be harnessed to ensure the continued survival and evolution of what remains of the world's freshwater fish fauna. Cambray & Pister (2002) noted that public support was essential and there must be education and extension programmes that become part of the total scientific study programme (as is currently happening in some of the work for European Union projects, eg Collares-Pereira et al., 2002).

Loss of aquatic biodiversity by introducing alien species solely for the pursuit of pleasure needs to be urgently halted. In many cases host countries already had, or still have, good angling species. Even if they do not it is debatable whether a sport, mainly for pleasure, should be the cause of the loss of biodiversity in the 21st century.

The present *status quo* of alien sport fish availability drives angler expectations. Leopold (1949) wrote 'To promote perception is the only creative form of recreational engineering'. It is now time to promote the perception that conservation of biodiversity is more important than introduction of aliens for fishing pleasure.

If scientists with the facts, who work on freshwater fish species, do not encourage public awareness then who will?

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Integration of research and management in optimizing multiple uses of reservoirs: the experience in South America and Brazilian case studies

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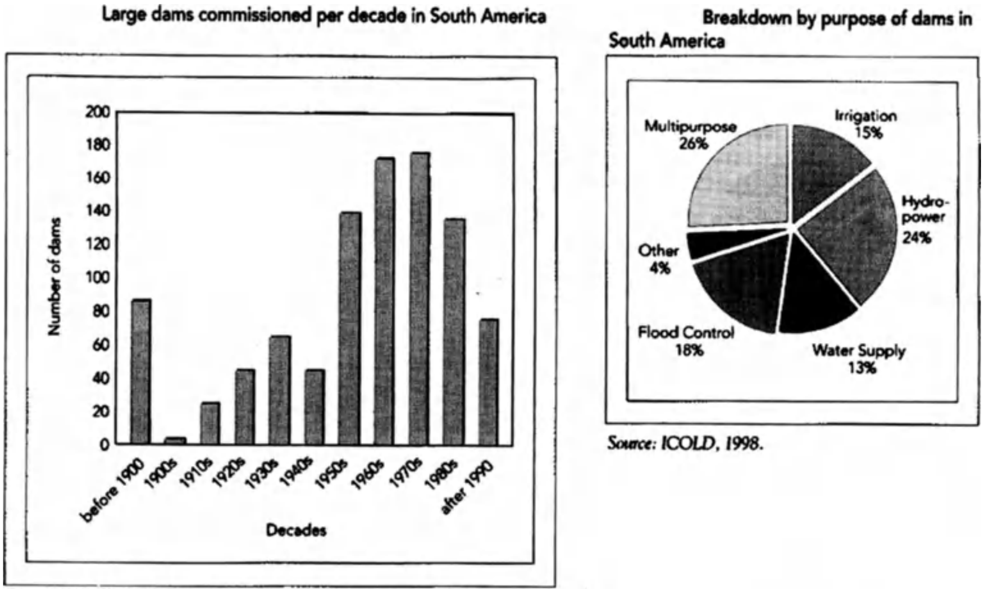
Abstract

The construction of large reservoirs in South America and particularly in Brazil has intensified within the last 50 years. Built up primarily for hydroelectricity production, these artificial ecosystems now serve purposes such as: water storage for public use; fisheries and aquaculture; recreation; tourism, and irrigation. These artificial ecosystems were also built up with the purpose of enhancing the regional development. These activities produce multiple impacts among which are eutrophication, a serious problems with various ecological, economic, and social consequences. Basic studies on reservoirs have identified their main ecological characteristics and described some of their fundamental mechanisms of functioning. This information cover: factors involved in reservoir complexity, such as spatial scale, vertical and horizontal heterogeneity, temporal variation at several time scales and, in some cases, watershed/reservoir relationships, and the impact of non-point and point sources of nutrients. Numerous studies have been dedicated to describing aquatic biota, its relationship with environmental factors, and the impacts of degradation/pollution/ eutrophication on biodiversity. Research priorities have been established mainly by limnologists in response to needs identified by environmental impact assessment. Due to the pressure of multiple uses, population growth, and economic factors, aquatic scientists now face a number of questions being posed by managers and engineers. These questions relate to a wide range of practical problems, the solutions to which depend on accumulated data related to the structural complexities and function mechanisms previously referred to. These problems can be divided into three main groups: (i) eutrophication processes and their characteristics, and water quality control; (ii) impact of fisheries, aquaculture, and exotic species introduction on the biota and the water quality; (iii) sustainable development of reservoirs and optimization management of their multiple uses. Predictive capabilities required in the face of these problems should be based on modeling development and intensive use of data-bases resulting from long-term studies on reservoirs. The predictions thus made possible would involve economic factors and the costs of recovery of eutrophic reservoirs. The experience of reservoir research and management in South America and in Brazil is, probably part of a world wide tendency on reservoir research and development with the aim to optimize multiple uses. Based on the cooperation of limnologists and engineers, ecosystem management models must eventually be applied at the watershed / reservoir level, where demands on the part of managers and decision makers will certainly promote a **predictive, integrated, and adaptive approach,** based on fundamental research.

Introduction

Reservoir construction in South America was intensified during the last 50 years of the 20th Century. Particularly in Brazil, large-dam construction in the 1960s and 1970s strongly interfered with river functioning and the hydrological cycles, producing many changes in these cycles and in the biodiversity related to the rivers, especially in the Paraná – La Plata basin. Several research papers (Junk & Melo, 1987; Tundisi, 1994; Agostinho et al., 1999; Tundisi et al., 1999; Straskraba & Tundisi, 1999) showed the changes pro-



Source: ICOLD, 1998. Note: Rates of dam commissioning in the 1990s are underreported.

Source: ICOLD, 1998.

Figure 1. Large dams commissioned per decade in South America. Breakdown by purpose of dams in South America.

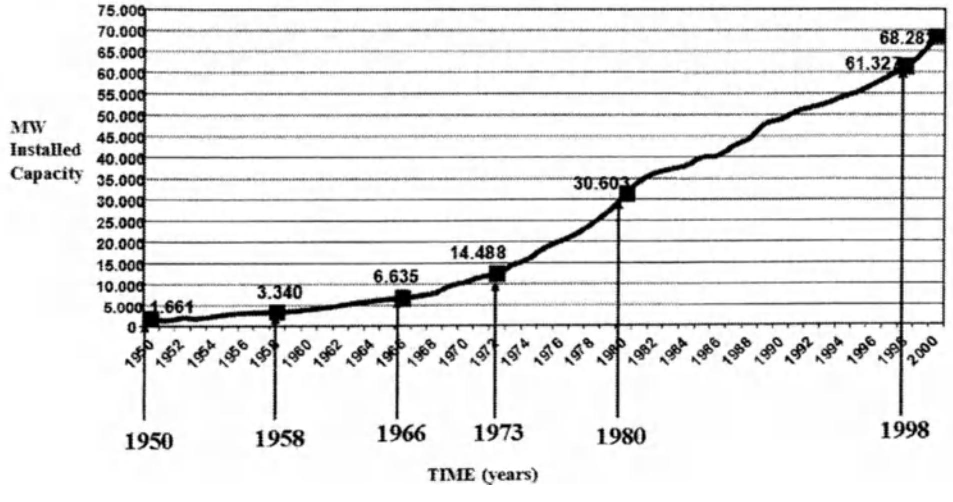


Figure 2. Evolution of installed capacity in Brazil (MW) from 1950 to 1998.

duced in the natural systems by reservoir construction and also the multiple uses which they brought about or enhanced. The positive and negative impacts of large dam construction on such a broad scale has been described in detail elsewhere (Tundisi et al., 2002). Figure 1 shows the decadal commission of large South American dams and a breakdown according to their use (ICOLD, 1998).

Figure 2 shows the evolution of hydroelectric capacity strictly for energy production in Brazil from

Table 1. Argentine Reservoir Storage and Installed Capacity

Up to year	Number	Reservoir storage (Hm^3)	Installed Capacity (Mw)
1900	4	142	---
1930	6	187	---
1940	10	802	11
1950	32	1.854	172
1960	45	2.396	249
1970	64	5.949	541
1980	87	82.965	4011
1990	96	88.856	5743

Calcagno (1994).

1950 to 1998. Many more smaller reservoirs (up to 100 million m^3) were built up from 1900 to 2000 (150 000 for other uses such as water storage, fisheries and irrigation).

Table 1 describes the argentine reservoir storage and installed capacity. Reservoirs in South America and in Brazil are located at a wide range of latitudes, from the northern tropics to the southern subtropical or near-temperate systems (Henry, 1999) (Table II). These constructions present a broad gamut of morphometric characteristics, areas, and maximum depths. Figure 3 shows the surface seasonal temperature variation of some of them located at a range of latitudes, in Brazil.

In this paper, the authors describe and discuss the evolution of multiple uses of South American reservoirs, emphasizing the Brazilian ones, and how the demands created by practical management problems reoriented pure basic towards problem-solving basic research. This is given as an example of recent changes in the research direction from basic Limnology to its applications. Despite the regional example, it could be part of a world wide tendency in research and management of aquatic ecosystems.

Evolution of multiple uses of reservoirs in South America and Brazil (nineteenth to the twenty-first century)

In the early period of reservoir construction (1890–1940) the following objectives were established for reservoir construction in Brazil: storage of water for drinking purposes, fish stock for biomass production, storage of water for agricultural purposes and small scale hydroelectricity production. The reservoirs were relatively small with less complex morphometry and volumes of up to 100 million m^3.

Considering reservoir size distribution and uses in the early reservoir-construction period, management priorities were relatively simple until the 1940s. Water quantity had to be maintained for hydroelectricity production, irrigation, and fish stocks; water quality was essential for drinking water reservoirs.

In the second reservoir-construction phase, size, volume, and morphometric complexity increased; uses multiplied, extending to large-scale hydroelectricity production, irrigation, and fishery.

In the final decades of the 20th century, a vast gamut of activity was taking place on these reservoirs, including: fish stocking, aquaculture, recreation, tourism, water transfer, drinking water storage, and industrial water recycling. Navigation and extensive transportation of grains, wood, cattle, and sugarcane were also introduced during this period. Reservoir volumes increased enormously, reaching 10–20 billion m^3 or even more in special cases.

The advances in limnological studies of reservoirs

Early reservoir studies (1910–1970) concentrated on specific areas such as taxonomy and geographic distribution of aquatic organisms; fish and fisheries; fish stock; water quality and sanitary engineering for drinking water reservoirs; and descriptive limnology related to species distribution and hydrobiological problems (Branco, 1999). Interestingly enough, early descriptions of zooplanktonic species were made by researchers in the health area.

Large scale reservoir construction stimulated very intensive development of limnological research directed mainly at the reservoir structure and function. This research period resulted in an active reservoir Science, that proved extremely useful in diagnosing reservoir impacts, eutrophication processes, and the relationships between the reservoir cascades and their watersheds. Some major advances promoted by research in reservoir limnology are described. This is a non exhaustive list of the existing bibliography.

Some major advances in limnology and ecology of reservoirs in South America and Brazil (1970–2000)

- Comparative reservoir studies

Tundisi, 1981.

- Thermal structure, circulation patterns

Arcifa et al., 1990; Henry & Tundisi, 1988; Henry, 1995, 1999.

- Temporal and spatial fluctuations

Heide, 1982; Hernandez et al., 1988; Henry, 1992, 1993, 1999.

- Biogeochemical cycles in reservoirs

Greenhouse gas emission studies Rosa et al., 1994; Fearnside, 1995; Abe et al., 2001; Bitar & Bianchini, 2002.

- Reservoir biodiversity patterns (zooplankton & phytoplankton, fish communities) Moreno, 1996; Agostinho et al., 1999, 1997, 1999; Nogueira et al., 2002.
- Primary production of phytoplankton short term changes; integration with hydrological cycle Tundisi, 1993; Matsumura-Tundisi et al., 1997; Rocha et al., 1997, 1999; Henry, 1999.
- Studies on reservoirs cascades

Rocha et al., 1999; Barbosa et al., 1999; Guntzel, 2000.

- Loading of watersheds to reservoirs; retention time as ecological factor

Tundisi et al, 1993, Braga et al., 1998; Straskraba, 1999; Straskraba & Tundisi, 1999.

- Application of remote sending and GIS to reservoir management.

Novo et al., 1995.

- Reservoir aging and reservoir colonization

Heide, 1982; Agostinho et al., 1992, 1995, 1999.

- Fish stock, fisheries

Borghetti et al., 1999; Quiros, 1999; Bechara et al., 1999.

- River–reservoir interactions

Bonetto, 1994.

- Reservoir circulation patterns

Eiger, 1999.

- Zooplankton diversity in reservoirs

Rocha et al., 1997; Lopez et al., 2001; Matsumura-Tundisi & Tundisi, 2002

- Fish biodiversity and spatial patterns in reservoirs

Amaral & Petrere, 2001.

In general, these research topics encompassed the following concepts/questions:

- Succession of terrestrial and aquatic systems, and the impacts of filling.
- The pulse concept applied to reservoirs.
- The ecotone concept and the mosaic.
- The concept of connectivity.
- Spatial heterogeneity in reservoirs.
- Dynamic interfaces.
- The forcing functions concept.
- Networks.
- Community structure and population ecology.
- Reservoir aging and colonization.
- Upstream/downstream interactions.

Complexity of reservoirs and management

The needs for reservoir management were certainly very much increased after the second phase of large scale reservoir construction: the size, morphometry, and multiple use of reservoirs, promoted these artificial ecosystems to the scale of enormous complex systems, with a vast range of uses and varying operational processes which interfere with the ecological and limnological characteristics upstream and downstream.

Reservoirs have, in comparison with lakes, high watersheds area/water body area, shorter but varying retentions times, a rapid ageing process related to watershed uses, high capability to retain organic and inorganic matter (Straskraba, 1998; Straskraba & Tundisi, 1999).

Reservoirs have **unidirectional changes of limnological variables**, a relatively **extense transitional zone,** and **several outlets** and **off takes** that can produce extensive changes in the reservoir functioning (Straskraba et al., 1993; Straskraba, 1997). Therefore the management of the reservoir ecosystem or reservoir cascades is much more complex than that of lakes due to these morphometric, functional and operational characteristics. There is a switch of the behavior of the system as the environmental conditions are varied and this increases complexity and interferes with the management technology and structural and non-structural actions for management. Thus, the management priorities of the reservoirs became diversified and more complex: it was necessary to improve the capacity to optimize multiple uses that were imposed on the reservoirs by the needs of society and to develop economic and social mechanisms for improvement of the regions after reservoir construction. A new reservoir in a river basin implies in a change in the hydrosocial cycle, that is the social cycle adapted to the water cycle, of the human population in the basin (Tundisi, 1989; Tundisi et al., 2002).

The use of limnological information in reservoir management

Due to the needs of implementing measures recommended by environmental impact assessment of reservoirs, many limnological studies were developed in the rivers prior to the reservoir construction. Environmental changes were anticipated through studies such as those developed on Itaipu reservoir and other ecosystems by Agostinho et al. (1992, 1994 a, b) and Tundisi et al. (2002). Prognostication on reservoir aging and the impacts of watershed uses became an important tool in reservoir management and operation (Tundisi et al. 1993, 1999). In addition, the linkages

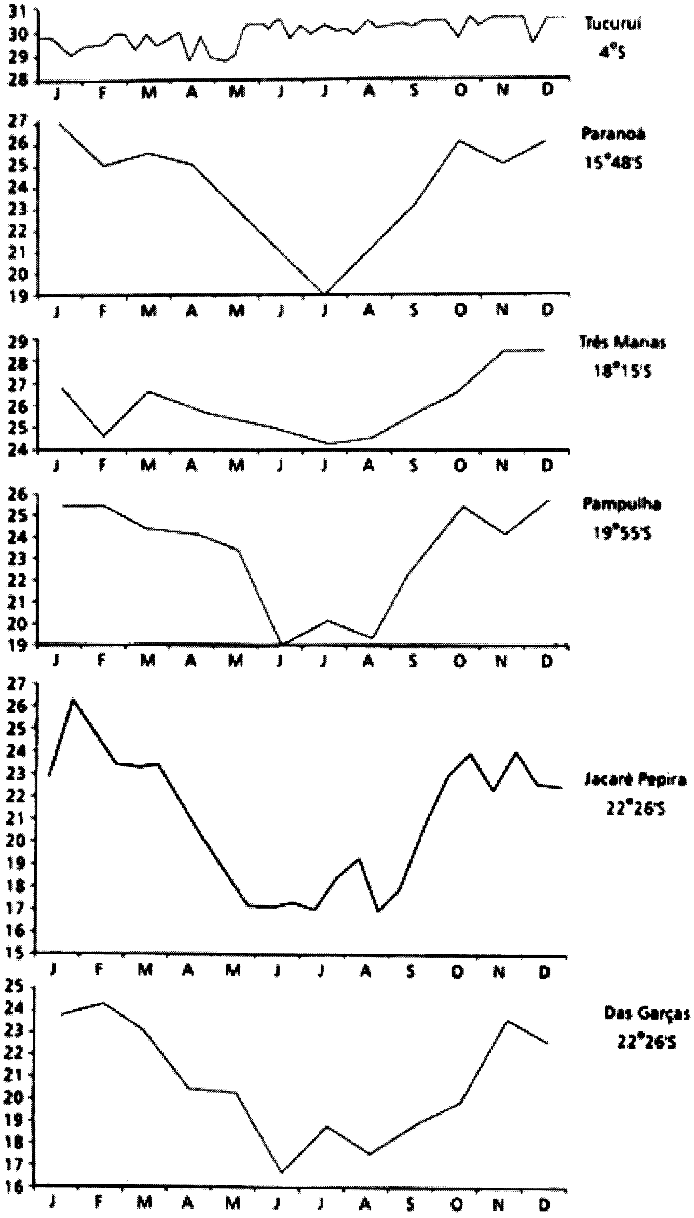


Figure 3. Annual cycle of surface water temperature for six reservoirs in Brazil (Henry, 1999).

Table 2. Morphometric parameters and theoretical residence times of some brazilian reservoirs

Morphometric parameters and theoretical residence times of Brazilian reservoirs

Reservoir	Latitude	Longitude	Elevation m.a.s.l.	Area (km)^2	Z (m)	Zmax (m)	Theoretical Residence Time (days)
Tucurui	3° 43′ S	49° 12 W	72	2430	17.3	75	51
Boa Esperança	6° 45′ S	43° 34′ W	304	300	∼35	196	
Paranoá	15° 48′ S	47° 75′ W	1000	40	14.3	38	300
Três Marias	18° 15′ S	44° 18′ W	585	1120	6.8	∼30	29
Pampulha	19° 55′ S	43° 56′ W		2.4	5.0	16	120
Volta Grande	20° 10′S	48° 25′W		222	10.2		25
Monjolinho	22° 01′ S	47° 53′ W	812	0.05	1.5	30	–10
Dourada	22° 11′ S	47° 55′W	715	0.08	2.6	∼6.3	
Jacaré	22° 18′ S	47° 13′ W	600	0.003	0.9	∼2.2	11
Jacaré-Pepira	22° 26′ S	48° 01′ W	800	3.7	3.0	12	
Jurumirim	23° 29′ S	49° 52′ W	568	446	12.9	40	322
Das Garças	23° 39′S	46° 37′W	798	0.09	2.1	46	69
Itaipu	25° 33′ S	54° 37′ W	223	1460	21.5	140	40

Henry (1999).

between reservoir typology, water quality, biodiversity and reservoir operation were described in several papers such as Agostinho et al. (1999), Barbosa et al. (1999) and Tundisi & Straskraba (1999). Thus, the bulk of knowledge produced in the last 30 years turned into a tool in managing the artificial ecosystems, including watershed input, and in elaborating the new technology for reservoir construction and operation. Reservoir management in South America and in Brazil improved considerably during the last decade of the 20th century as a consequence of the increasing flow of information from scientific research. As an example the management of drinking water reservoirs in the Metropolitan Region of São Paulo was more effective due to the studies on phytoplankton ecology and biogeochemical cycles (Beyruth, 2000).

The conceptual basis for management procedures has been considered in many case studies, pilot projects and has been applied for single reservoirs or reservoir cascades (Agostinho et al., 1994; Boneto, 1994; Petrere, 1996). These conceptual frameworks, were placed as:

- Control of pulses.
- Control of succession.
- Maintaining/restoring biodiversity.
- Control of excess nutrients from the watersheds (non point and point sources of nitrogen and phosphorous).
- Maintaining and controlling the interaction upstream – downstream.

Figure 4 shows as an example the change in fish fauna in Itaipu reservoir after the construction of the impoundment. Several ichtyological studies in many reservoirs were fundamental altogether with the limnological studies to understand reservoir complexity and to provide inputs for their management including the operations.

The integration of Limnology with reservoir management has been discussed at length in South America, particulary for the La Plata basin a international watershed shared by 5 countries (UNCRD, 1994). The problems of health impacts of reservoir construction, fish stocks and fisheries, theoretical and operational issues in resettlement processes, were discussed as a potential for new management strategies that should be based on fundamental ecological and limnological

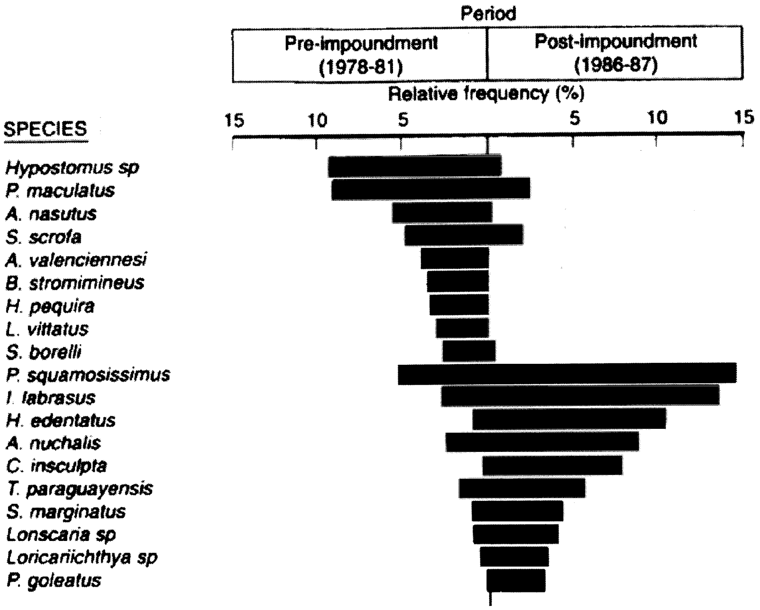


Figure 4. Relative frequency of the 10 principal species of fish before and after Itaipu impoundment. Source: from Agostinho et al. (1994a).

research (Bartolomé, 1994; Oldani, 1994; Ronderos, 1994; Agostinho et al., 1994a).

Multiple uses and management: linking theoretical and operation approaches

As discussed by Kennedy (1999), the linkages found between construction and operation of reservoirs allowed insights into their limnological character and mechanisms of functioning. As multiple uses and reservoir morphometric and functional complexity increased, new questions were being posed, the answers to which depended upon further studies. In this way, within the last 10 years, limnological research has come to be driven by management needs. Thus, it is no longer enough to understand reservoir limnology. What must also be grasped is its interaction with the watershed from the early phases of construction to the final operational stage, with all the functioning mechanisms thus entailed. In addition, the ecological dynamics of the reservoirs have to be understood in relation to watershed uses and impacts (Straskraba et al., 1993; Tundisi et al., 1999). The necessary new policies and changes required for reservoir multiple uses must now be considered. They include:

- Protection of ecosystem processes;
- integration of biogeophysical, ecological, economic and social data in an information system and a data bank;
- maintenance of sustainability of multiple uses through adaptive management;
- development of a permanent system of water quality monitoring in real time as a watershed activity sensor;
- preparation of inter and multidisciplinary teams to develop integrated and integrative management strategies.
- Promotion of predictive capabilities for reservoir management.

Figure 5 shows the new paradigmas for reservoirs management in order to link basic research to management.

The design of new management strategies has been intensified by the implementation of social and environmental assessment recommendations as well as those drawn from economic and financial analyses of reservoir construction and their positive and negative impacts (WCD, 2000) (Figs 6 and 7).

5b.

Figure 5. The new perspectives for management and the changes in the strategies posed by the demands (Figure 5b modified from Ayensu et al., 1999).

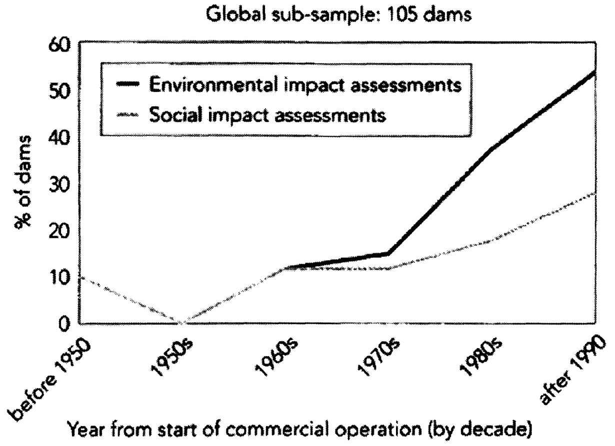


Figure 6. Trends in the implementation of environmental and social assessment of reservoirs (WCD, 2000).

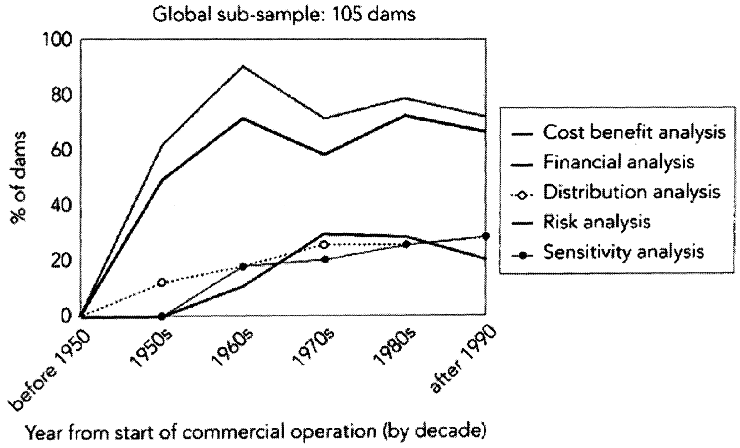


Figure 7. Trends in the implementation of economic and finantial analyses (WCD, 2000).

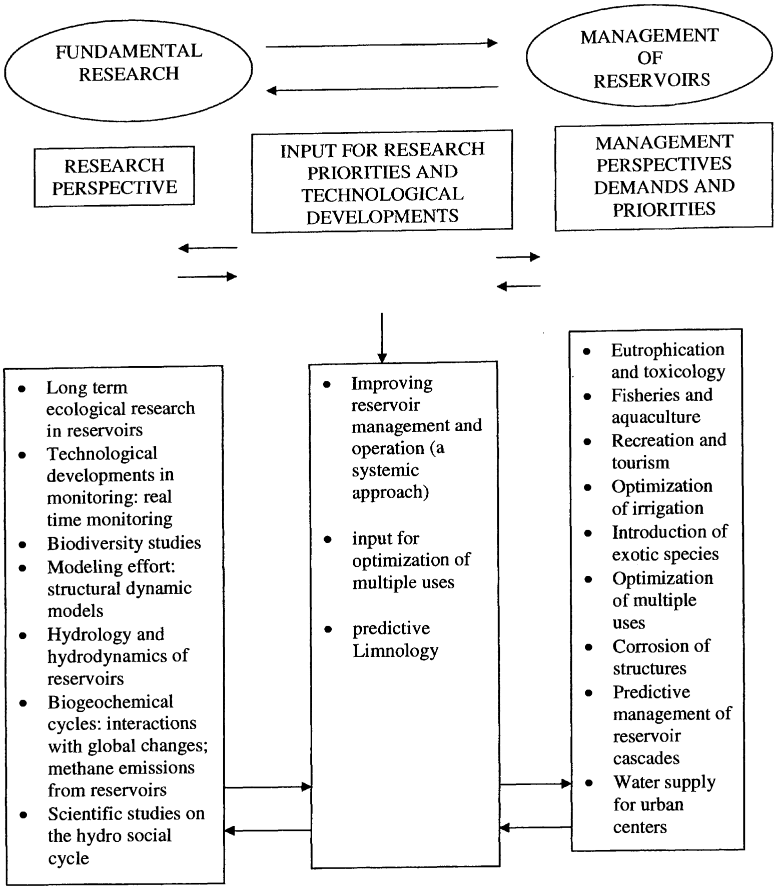


Figure 8. Present needs for the management of reservoirs in South America in general and Brazil in particular, and the required inputs from the fundamental research.

Conclusions

Throughout South America, and particularly in Brazil, recent management strategies for optimization of multiple uses of reservoirs have been formulated, based on information yielded by relevant economic and social assessments, in response to pressures, originating in public and private sectors, to exploit the opportunities which these artificial ecosystems create. At the same time, management initiatives and needs have posed new questions for limnologists and stimulated further basic research directed to problem solving in reservoir ecology (Braga et al., 1998).

Results of the management-driven limnological research currently enable managers to choose among many solidly based options for watersheds and sustainable reservoir development. The research itself is also vital in the training of an entire new generation of limnologists as they continue the quest for knowledge about reservoir ecosystems.

Somlyody et al. (2001) emphasized the new set of problems and challenges that are posed to water resources planners and managers. They include an extensive list of water uses that in South America, and particularly in Brazil, are centered on reservoirs: irrigation and municipal water demands, navigation, recreation, sediment control, flood control, insect and water borne disease control, hydroelectricity generation, industrial processing and cooling, tourism, urban runoff control. All the impacts have economic and social consequences and have to be considered in the fundamental research that will support the management strategies and in the integrated management. A better predictive and adaptive management strategy is based on good predictive Limnology of reservoirs as stressed by Straskraba et al. (1993), Straskraba (1995) and Tundisi et al, 2003, in press. The demands for management also can indicate what basic data are lacking that will be useful to prepare the plans for the specific watershed/reservoirs (Menshutkin and Klekowshi, 2001; Rocha et al., 2002)

Present needs for the management of reservoirs in South America in general and Brazil in particular, and the required inputs from the fundamental research, in order to develop adequate management strategies are presented in Figure 8.

To maintain the sustainable use of reservoirs, a tightly coupled interaction between fundamental research and reservoir management is needed. This is shown in Figure 8, where the research perspectives and their inputs into management actions are demonstrated. This is probably the most prominent way in attaining environmental quality, conservation of aquatic biodiversity and the guarantee of multiple uses of these very important water bodies.

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Sinking properties of some phytoplankton shapes and the relation of form resistance to morphological diversity of plankton – an experimental study

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Abstract

Form resistance (Φ) is a dimensionless number expressing how much slower or faster a particle of any form sinks in a fluid medium than the sphere of equivalent volume. Form resistance factors of PVC models of phytoplankton sinking in glycerin were measured in a large aquarium ($0.6 \times 0.6 \times 0.95$ m). For cylindrical forms, a positive relationship was found between Φ and length/width ratio. Coiling decreased Φ in filamentous forms. Form resistance of *Asterionella* colonies increased from single cells up to 6-celled colonies than remained nearly constant. For *Fragilaria crotonensis* chains, no such upper limit to Φ was observed in chains of up to 20 cells (longer ones were not measured). The effect of symmetry on Φ was tested in 1–6-celled *Asterionella* colonies, having variable angles between the cells, and in *Tetrastrum staurogeniaeforme* coenobia, having different spine arrangements. In all cases, symmetric forms had considerably higher form resistance than asymmetric ones. However, for *Pediastrum* coenobia with symmetric/asymmetric fenestration, no difference was observed with respect to symmetry. Increasing number and length of spines on *Tetrastrum* coenobia substantially increased Φ. For a series of *Staurastrum* forms, a significant positive correlation was found between arm-length/cell-width ratio and Φ: protuberances increased form resistance. Flagellates (*Rhodomonas, Gymnodinium*) had a $\Phi < 1$: they sank faster than the spheres of equivalent volume. *Ceratium* ($\Phi = 1.61$) proved an exception among flagellates: in most forms tested in this study (ellipsoid flagellates, *Staurastrum* forms with no or very short protuberances, and *Cosmarium* forms), $\Phi > 1$. The highest value ($\Phi = 8.1$) was established for a 20-celled *Fragilaria crotonensis* chain. Possible origin of the so-called 'vital component' (a factor that shows how much slower viable populations sink than morphologically similar senescent or dead ones) is discussed, as is the role of form resistance in evolution of high diversity of plankton morphologies.

Introduction

After Antoni van Leeuwenhoek (1632–1723) constructed his first microscopes, morphological variability, diversity and the general *beauty* of planktonic organisms have attracted generations of taxonomists and plankton ecologists. The huge diversity that we can see under the microscope evolved during the millennia of life on planet Earth and the main evolutionary driving force was certainly something other than to fascinate scientists.

Planktonic organisms are usually small and they have short generation times. Typical temporal patterns include irregular fluctuations around a more or less constant level, increases and decreases over long periods, cyclic oscillations and occasionally explosive bursts by populations normally existing at low levels (Lampert & Sommer, 1997). The net growth rate (r) of a phytoplankton population can be expressed as $r = \mu - \lambda$, where μ is the gross growth rate and λ symbolizes losses. The latter, λ, includes various sources of loss, such as predation (grazing by the zooplankton), physiological mortality due to aging, the mortality caused by parasites, washout loss to the outflow and settlement to the bottom sediments. These loss factors are rather universal for most biota, except washout and sedimentation are quite specific for plankton. The velocity of a particle sinking in a fluid medium can be described by the Stokes equation:

$$v_s = \frac{2gr^2(p' - p)}{g\eta\Phi},$$

where v_s [m s^{-1}] is the sinking velocity, g [m s^{-2}] is the gravitational acceleration, r [m] is the radius of the sinking spherical particle, p' is the specific gravity of the sinking particle [kg m^{-3}], p is the specific gravity of the fluid medium [kg m^{-3}], and η [kg m^{-1} s^{-1}] is the viscosity of the medium. Φ is the form resistance factor, which is a dimension-less number and it expresses the factor by which the sinking velocity of the particle differs from that of a sphere of identical volume and density. If a planktonic species evolves towards minimizing its sinking losses, it has three options: it may decrease its body size (however, to do so is to increase the risk of being grazed by zooplankton), it may decrease its specific gravity (examples include gas vacuoles of cyanoprokaryota and the accumulation of oil-droplets as storage product), or it may increase its form resistance.

The validity of the Stokes equation was tested in experiments with simple geometrical forms made of metal and sinking in viscous oil. It was shown that each shape tested, except the tear-drop, sank more slowly than the equivalent sphere (McNown & Malaika, 1950). For phytoplankton species generally, form resistance factors between 0.94 and 5.49 were established (Reynolds, 1984). The method of most experiments aiming establishment of Φ was measuring sinking velocity of a laboratory culture of a given species and comparison of the measured values to those estimated from the Stokes-equation for spheres.

The aim of the present study was to measure form resistance of the most common phytoplankton forms and/or morphological types, using artificial PVC models of algal forms in a laboratory experimental aquarium containing a viscous fluid.

Experimental design, material and method

Two basic criteria were kept in mind when designing the experimental aquaria to study sinking velocities. (i) Sinking particles generate turbulence as they move through water, whereas the influence of turbulence on sinking velocities needed to be excluded. Therefore, the experimental aquarium had to be both wide (to avoid wall-effects) and long (to allow as long way to sink as possible for enhancing accuracy of the measurements). (ii) As the density difference between water (max. 1 g cm^{-3}) and phytoplankton is rather small (the heaviest diatoms have densities up to 1.29 g cm^{-3}; for other groups than diatoms the 1.04 g cm^{-3} is characteristic [Reynolds, 1984]), we needed to minimize the density difference between the medium and sinking particles.

The experimental system consisted of two aquaria. The inner was 0.6 × 0.6 × 0.95 m and the outer was 0.7 × 0.7 × 0.95 m (vertical dimensions were the highest; thickness of the glass was 1.0 cm). Since the densities of most fluid media are highly temperature-dependent, the outer aquarium was intended to moderate the inner one. However, this precaution proved to be unnecessary since the experiments themselves could be carried out rapidly (even with many repetitions) and it proved to be easier to establish calibration curves for any given ambient temperature than to operate a heating system (+ stirring pumps) in the external aquarium. A simple mosquito net fixed on an iron-frame was placed at the bottom of the aquarium for recovering settled particles.

Concerning the medium and the material for the artificial algae, the mineral oil + clay ceramics seemed initially to be an optimal combination but we were compelled to reject it. Most mineral oils that were sufficiently viscous were not adequately transparent, so requiring the installation of electronic sensors to record settlement times. Moreover, it seemed both problematic and unappealing to recover particles from the bottom of the aquarium prior to making replicate measurements with the same particle. For these reasons, we decided to use glycerine as the fluid medium (86.5%, P 20–25 °C = 1.2271 – 1.2214 g cm^{-3}; Perry, 1969).

For the model algae used in the first set of experiments, we used PVC-U (P' = 1.37 g cm^{-3}). This material is a hard plastic and ideal for machine turning. Thus, the density difference between the sinking particle and the medium was $\sim$ 1.12 which falls into the range of the density difference between algae and water. Algal shapes (spheres, rods, *Astrionella formosa* and *Fragilaria crotonensis*) were machine-turned and used for the experiments. Later, we had to accept that machine turning, although capable of producing near-identical 'cells', is not appropriate for fashioning the complex forms of *Staurastrum* or *Ceratium*. For making such forms, a PVC-based (Plastillin-like) modeling material (HAVO B.V. POB 320, 3850 AH Ermelo, Holland) was used. This material is com-

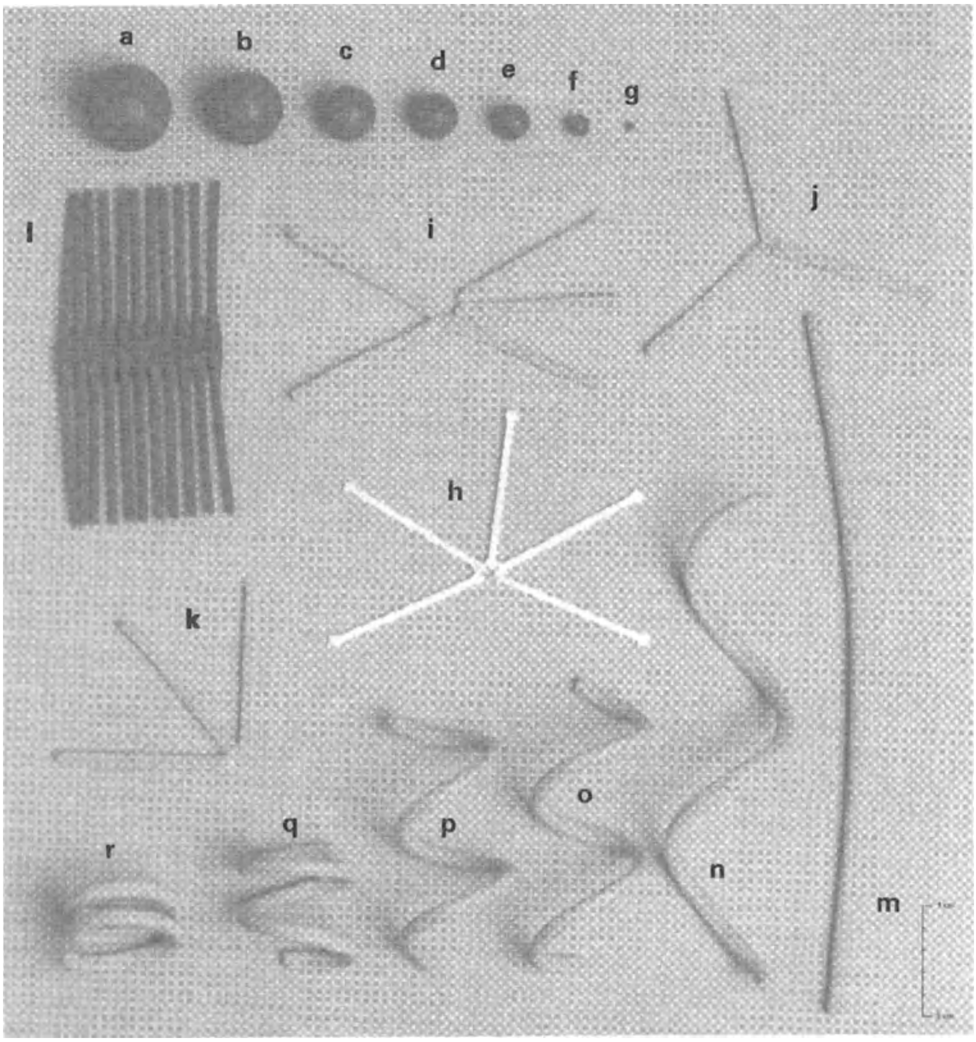

Plate I. (a–g) example of series of spheres used for calibration; (h–i) *Asterionella* colonies with 5 cells and diifferent symmetry; (j) symmetric *Asterionella* colony with 3 cells; (k) asymmetric *Asterionella* colony with 3 cells; (l) *Fragilaria crotonensis* chain with 11 cells; m–r: filaments coiled at different degree. Forms a–h and l were made of PVC-U, the others of modelling material. Scale bar spans 5 cm.

monly found in toy- or hobby-shops; target users are children who make flowers, animals, etc. from it because it is readily malleable. Then they can be put into kitchen-oven where they harden (without losing its original bright colors). In our experiments, algal shapes were heat-treated (130 °C) in a kitchen oven for 30 min. The specific gravity of this material was established as 1.84 g cm^{-3} so that its density ratio to glycerine is 1.50. This is higher than the ratio between phytoplankton and lake water, however, we had to compromise. Spines for *Tetrastrum*-like forms were cut from thin but hard transparent plastic and were glued onto the forms. Photographs of some artificial algae are provided on Plates I–III.

Sinking times of each particle were measured in many replicates and the averages were used for the

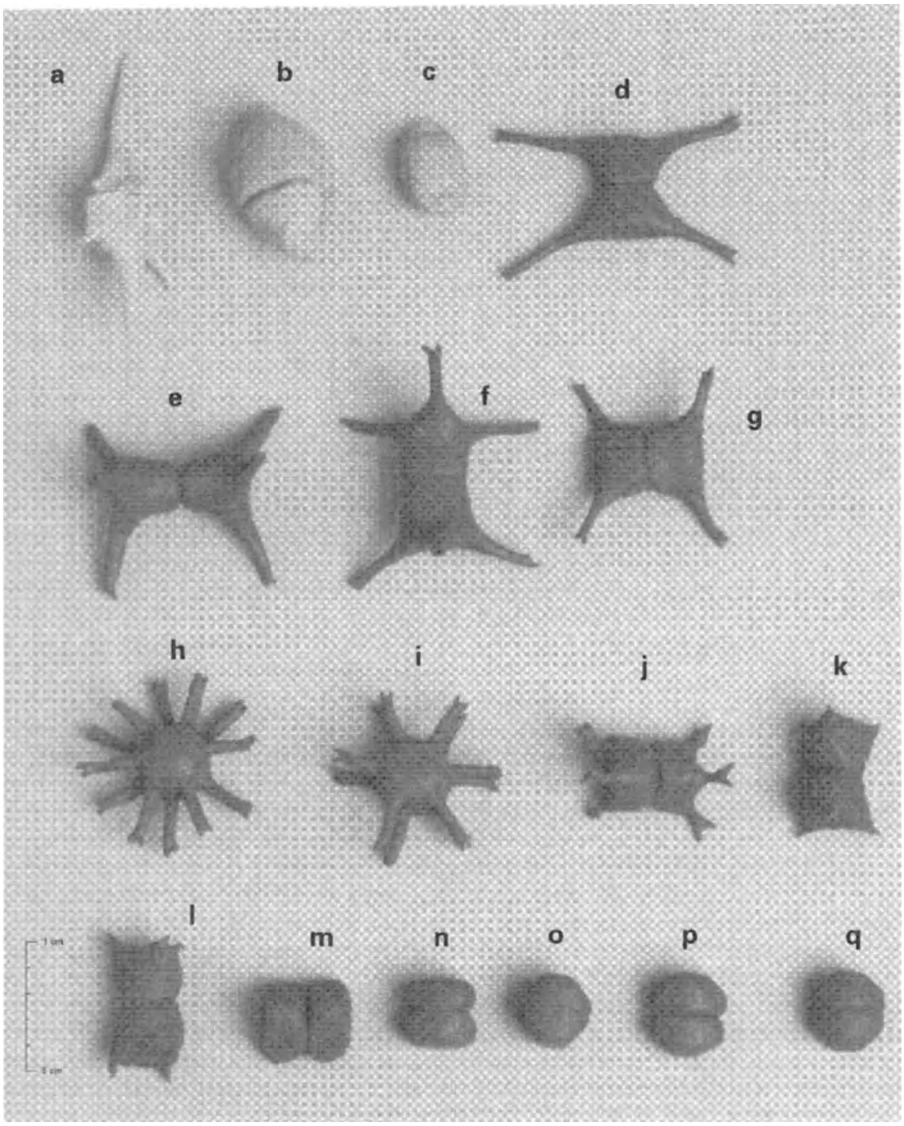

Plate II. (a) *Ceratium hirundinella*; (b) *Gymnodinium helveticum*; (c) *Rhodomonas lens*; (d) *Staurastrum chaetoceras*; (e) *Staurastrum planktonicum*; (f) *Staurastrum luetkemuellerii*; (g) *Staurastrum tetracerum*; (h) *Staurastrum rotula* with alternating protuberances (natural form); *Staurastrum rotula* with ovalapping protuberances (artificial form); (j) *Staurastrum pelagicum*; (k) *Staurastrm lunatum*; (l) *Staurastrum avicula*; (m) *Staurastrum erasum*; (n) *Cosmarium bioculatum*; (o) *Cosmarium laeve*; (p) *Cosmarium ornatum* with smooth surface (artificial form); (q) *Cosmarium ornatum* with papillate surface (natural form). Each form was made of modelling material. Scale bar spans 5 cm.

further calculations. Standard deviation of sinking velocities were usually moderate.

The volumes of the algal models were measured gravimetrically and the equivalent spherical diameters were calculated. Spheres ($\Phi = 1$) of these diameters were made using the same modeling materials (PVC-U and modeling material; Plate I: a–g). These were weighed (precision: 10^{-3} g) and the diameters of non-

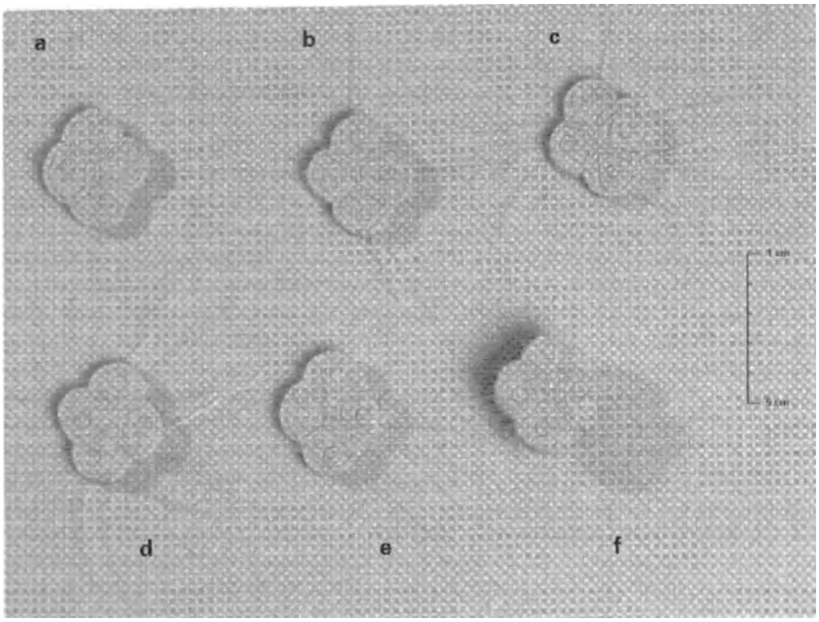

Plate III. Tetrastrum forms. (a) *T. glabrum* (no spines); (b) *T. hastiferum* with 4 symmetrically arranges spines; (c) *T. staurogeniaeforme* with ± symmetrically arranges spines; (d) *T. staurogeniaeforme* with asymmetrically arranges spines; (e–f) *T. hortobagyi* like form with different number of short, asymmetrically arranges spines. Each form was made of modelling material. Scale bar spans 5 cm.

machined spheres were verified with calipers (having an accuracy of 10^{-1} mm). From these data, mass-diameter calibration curves were obtained (Fig. 1A). Sinking velocities of each sphere were then measured and data were expressed as function of their diameter (Fig. 1B).

The estimation of sinking velocity of the spheres equivalent to given algae having a more sophisticated form was carried out on the following way: We weighed the alga (arrow 1 on Fig. 1); projected the weight-to-diameter using the relationships given in Figure 1A (arrow 2 on Fig. 1). This diameter was transferred to diameter-sinking velocity relationship given in Figure 1B (arrow 3 on Fig. 1) and then was projected to the y axis (sinking velocity) of the curves like on Figure 1B (arrow 4 on Fig. 1). Such calibration curves were established for both materials (PVC-U and modeling material) and for each series of experimental measurements. The reason for this was not only to overcome the non-standardized temperature of the glycerin, for we had also to allow for the fact that glycerin is a hygroscopic material that is liable to alter its density and viscosity through time and, so, to uncontrolled experimental conditions.

Form resistance factors were then derived from dividing the estimated sinking velocity of the volume-equivalent sphere by the sinking velocity of the corresponding form actually measured.

Results

Cylinders

These experiments were carried out with PVC-U turned forms having a standard diameter of 5 mm and lengths varying between 1 and 40 mm. Their form is expressed as length/diameter (L/D) ratio, which ranged from 0.2 to 8. Experiments with these disc- (length < diameter) or rod-like (length > to >> diameter) shapes resulted in a significant relationship ($r = 0.79$; $n = 24$; $P < 0.1\%$) between L/D and Φ (Fig. 2A). However, at L/D < 2, the two variables appeared quite independent. The apparent scattering of data (Fig. 2A) was attributable to differences in sinking position. Cylinders did not change their sinking position while sinking. Those which were started in horizontal position sank more slowly than those

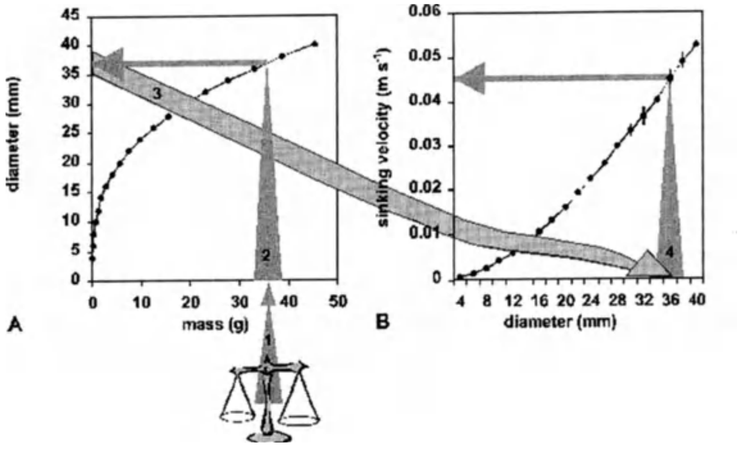

Figure 1. An example of mass-diameter (A) and diameter-sinking velocity (B) relationships for spherical particles. Grey arrows explain how sinking velocity of the sphere equivalent to a complicated form was established. First the complicated form was weighted (arrow 1), then the diameter of the equivalent sphere was calculated using relationship shown on Figure 1A (arrow 1). This diameter was transformed to Figure 1B (arrow 3) where from we got the appropriate sinking velocity (arrow 4).

set transversally and much more slowly than those set vertically. For reasons explained in the discussion, the effect of sinking position was not investigated in detail.

Effect of colony size on Φ: Fragilaria crotonensis, Asterionella

Asterionella (PVC-U forms; Plate I: h) colonies increased their Φ sharply in the range of 1 to 6 cells. Then Φ remained rather constant or even decreased slightly (Fig. 2B; records on this graph represent averages of altogether 154 individual measurements). It was apparent from the experiments that regular (8-celled) or close to regular colonies had the highest Φ. Up to 8-celled colonies, Φ of *Fragilaria crotonensis* (PVC-U forms; Plate I: l) were high but rather constant (Φ = 4.4–5.2; Fig. 2C; the number of individual measurements for this experiment was 180). For *Fragilaria* colonies consisting >8 cells, Φ increased rapidly with increasing cell numbers in the size range that was investigated (size of the aquarium did not allow construction of longer chains) reaching the highest Φ (8.1) that was recorded in any of our experiments.

Effect of coiling on Φ

For these experiments, 6 models (modeling material; Plate I: m–r) were used having exactly the same weights (volumes) and their 'straight' length was uniformly 295 mm. One of them remained slightly curved only, and the others were coiled to a differing degree. Coil width was 32 mm. For comparisons, L/D values were used where D was the width of the coil (32 mm) and length was the distance between the two ends of the filament. Similarly, as in the case of cylinders, starting position of the forms had a significant effect on Φ, however, in each case, the straightest filament had the greatest Φ and the most tightly coiled had the least (Fig. 2D).

Effect of symmety on Φ: Asterionella, Pediastrum, Tetrastrum staurogeniaeforme

When experimenting with PVC-U made *Asterionella* colonies with different cell numbers, we observed that Φ of (say) a 3-celled colony can be significantly influenced by the symmetry of arrangement of the three cells. Organisms used to test the effect of differing symmetry were *Asterionella* colonies consisting of 2–6 cells, *Pediastrum boryanum, Pediastrum duplex* and *Tetrastrum staurogeniaeforme.*

For *Asterionella* colonies, we expressed the degree of asymmetry as cumulative deviation from perfect symmetry (Plate I: i–k). For example: a 3-celled *Asterionella* colony is perfectly symmetric if each angle between the cells are 120°. For such an organism the cumulative deviation from perfect symmetry is 0 because |(120°– 120°)| +|(120°– 120°)|+ |(120°– 120°)| = 0°. If we have a 3-celled colony where the angles

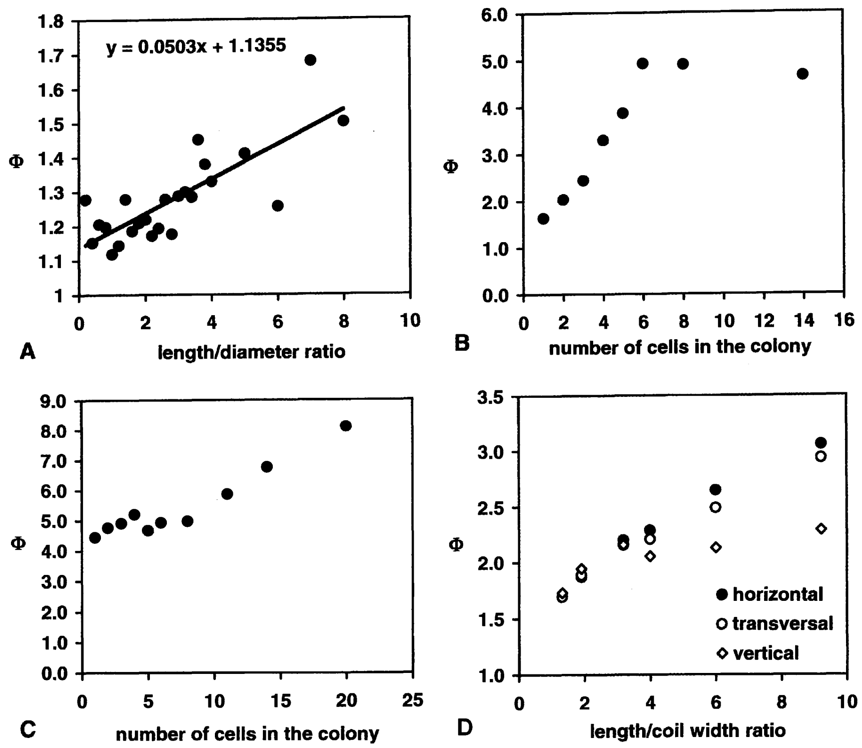

Figure 2. (A) Dependence of the form resistance factor (Φ) on the length/diameter ratio of cylindrical shapes (PVC-U). (B) Changes of the form resistance factor (Φ) depending on the number of cells in *Asterionella* colonies (PVC-U). (C) Changes of the form resistance factor (Φ) depending on the number of cells in *Fragilaria crotonensis* chains (PVC-U) and D: Changes of the form resistance factor (Φ) depending on degree of coiling expressed as length/coil width ratio (modelling material).

between the cells are 70°, 130° and 160° then the cumulative deviation from perfect symmetry can be calculated as |(70°– 120°)| +|(130°– 120°)|+ |(160°– 120°)| = 100°. Perfect symmetry for the tested colony sizes were: 2-celled – 180°; 3-celled – 120°; 4-celled – 90°, 5-celled – 72° and 6-celled – 60°. For each case in this experiment, form resistance decreased with decreasing symmetry of the tested colonies (Fig. 3), especially for colonies comprising only 2 – 5 cells. For colonies with >5 cells the relationship became increasingly neutral.

Pediastrum coenobia (especially *P. duplex*) have a number of holes (fenestrations) among the cells forming the coenobial disc. The arrangement of cells and fenestrations is usually quite symmetric, however deviations are common in nature. Asymmetry was introduced to *Pediastrum* forms by distributing holes symmetrically or highly asymmetrically on the two halves of the coenobium, but the proportion of the area fenestrated was kept constant. Form resistance in *Pediastrum* coenobia was ~2 (Table 1) and we could not get any statistically significant relationship between degree of asymmetry imposed by hole arrangement and Φ. For this reason data are not shown. Interestingly, even highly asymmetric forms maintained their initial sinking position despite considerable weight anomalies of the two opposing halves of the coenobia.

Tetrastrum coenobia (Plate III) with asymmetrically arranged spines (Fig. 4, open circles) had lower Φ than other coenobia having the same number/size of spines but with a symmetric arrangement (neighboring forms with close circles). These asymmetric forms had even smaller Φ than forms with no spines (form nr. 1 on Fig. 4). An interesting observation on very asymmetric *Tetrastrum* cells was that, regardless of the starting position, these forms reached a final orienta-

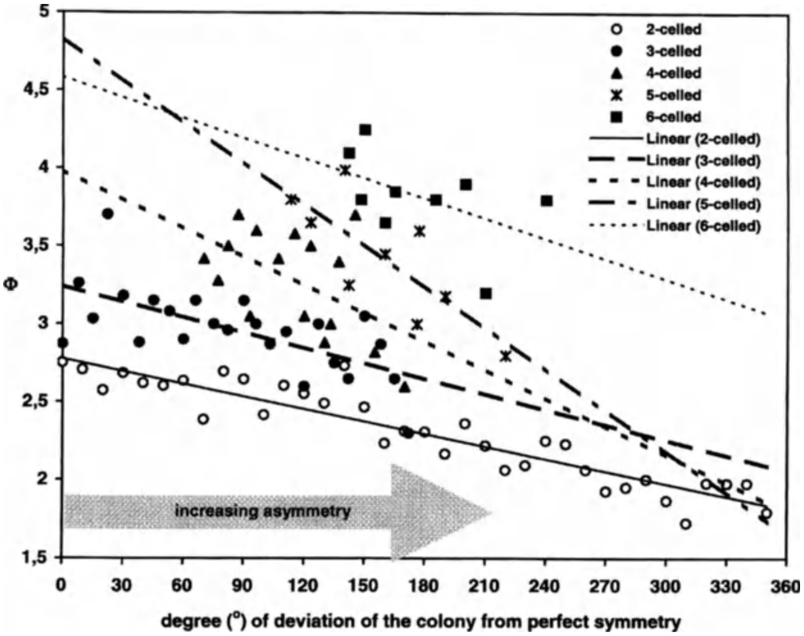

Figure 3. Changes of the form resistance factor (Φ) with increasing asymmetry (measured as cumulative degree (°) of deviation of the colony for perfect symmetry for 2-, 3-, 4-, 5- and 6-celled *Asterionella* colonies (modelling material). Trendlines according to linear regression are also indicated on the figure.

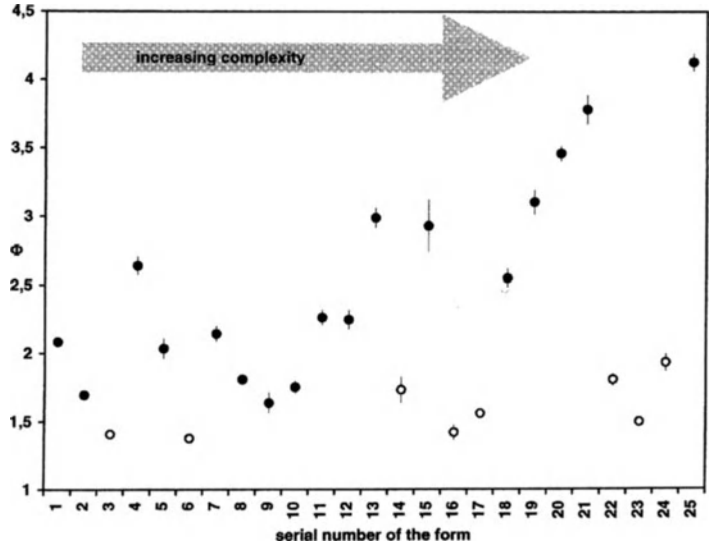

Figure 4. Changes of the form resistance factor (Φ) with increasing complexity. Serial number of modelling material made *Tetrastrum* forms are given on the x axis and they are arranged according to their increasing complexity concerning both numbers and length of attached plastic spines. Open circles represent forms with highly asymmetric arrangement of spines.

Table 1. Form resistance factor (Φ) and its standard deviation (SD) of some algal forms (modelling material)

Species	F	SD	Note
Pediastrum duplex	2.008	0.035	Horizontal sinking position
Pediastrum duplex	1.852	0.110	Vertical sinking position
Pediastrum duplex	1.936	0.037	Transversal sinking position
Pediastrum boryanum	1.908	0.041	Horizontal sinking position
Pediastrum boryanum	1.785	0.076	Vertical sinking position
Pediastrum boryanum	1.878	0.053	Transversal sinking position
Staurastrum rotula	1.424	0.027	Protuberances overlap; artificial form
Staurastrum rotula	1.459	0.019	Protuberances alternate; natural form
Staurastrum arctiscon	1.427	0.013	
Staurastrum planctonicum	1.578	0.321	
Staurastrum luetkemuelleri	1.487	0.026	
Staurastrum tetracerum	1.338	0.024	
Staurastrum chaetoceros	1.661	0.016	
Staurastrum avicula	1.058	0.018	
Staurastrum pelagicum	1.137	0.024	
Staurastrum lunatum	1.046	0.020	
Staurastrum erasum	0.886	0.018	
Cosmarium ornatum	0.837	0.003	With smooth surface
Cosmarium ornatum	0.856	0.009	With papillate surface
Cosmarium bioculatum	0.838	0.009	
Cosmarium laeve	0.810	0.005	
Ceratium hirundinella	1.609	0.170	
Rhodomonas lacustris	0.773	0.006	
Rhodomonas lens	0.676	0.004	
Gymnodinium helveticum	0.476	0.006	

tion very quickly (within the first 10 cm of sinking): with the spines on the upper side of the coenobium, dragged like a parachute.

The effect of spines (presence/absence, numbers, length, arrangement) on Φ: Tetrastrum, *desmids*

For these experiments, forms corresponding to morphologies of *Tetrastrum glabrum* (no spines; Plate III: a), *T. hastiferum* (few spines of different length; Plate III: b), *T. staurogeniaeforme* (Plate III: c,d) and *T. hortobagyi* (many spines with varying numbers and lengths; Plate III: e,f) were used. Complexity of these forms was understood to increase with both the number and the length of spines. Quantification of the x axis (Fig. 4) was rather problematic in this case, so data were arranged in order of complexity, identified by a serial number for the form.

Forms 1–10 (Fig. 4, × axis) were rather simple corresponding to *T. glabrum* (1) with no spines and *T. hastiferum* (2–10) with 4 spines, one on each of the four cells of the coenobium but of varying length. Forms 3 and 6 (open circles on Fig. 4) were different in having the 4 spines placed on separate cells but close to each other (this way appearing chiefly on one side of the coenobium). This artificial asymmetry (in nature, the spines are arranged at the greatest possible distance from each other) decreased the form resistance well below that of the naked (1) form. Nevertheless, at ≤ 4 spines, their lengths imposed no trend-like changes in the form resistance. Forms 11–25 corresponded to *T. staurogeniaeforme* or *T. hortobagyi* and these species were assigned because natural populations are very rich in the variety of detailed form (see Hindák, 1980, 1984; Komárek & Fott, 1983). Increasing complexity (in terms of both increasing number and length of spines) resulted in a significant (2–3 fold) increase of Φ unless the spines were arranged very asymmetrically (forms 14, 16, 17, 22, 23, 24; open circles on Fig. 4).

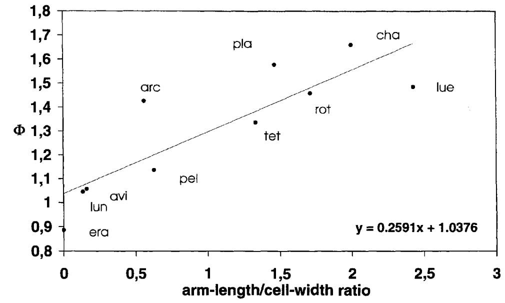

Figure 5. Dependence of the form resistance factor (Φ) on the arm-length/cell-width ratio of different *Staurastrum* forms (modelling material). Abbreviations – arc: *St. arcuatum*; avi: *St. avicula*; cha: *St. chaetoceras*; era: *St. erasum*; lue: *St. luetkemuelleri*; lun: *St. lunatum*; pel: *St. pelagicum*; pl: *St. planktonicum*; rot: *St. rotula* and tet: *St. tetraceras*.

Shapes of 11 *Staurastrum* spp. and 4 *Cosmarium* spp. (Plate II: d–q) were tested for form resistance (modeling material). Each of the *Cosmarium* spp. had $\Phi < 1$ which means that they sink faster than the volume-equivalent sphere (Table 1). *C. ornatum* was manufactured in duplicate: one with smooth surface (artificial form) and the other with papillate surface (natural forrm). This difference in morphology resulted in slightly higher form resistance in the papillate form. For *Staurastrum* spp., a significant correlation ($r = 0.86$; $n = 9$; $P < 0.1\%$) was found between the arm-length/cell-width ratio and Φ (Fig. 5).

While experimenting with desmids, the most striking phenomenon was their behaviour while sinking. Like in other experiments described above we took care of starting position of the cells. Whatever was the starting position, desmids (including the ones with low from resistance) turned to a position where the longest axis was horizontal. The distance required to perform this turn varied among cell shapes, but always within the first 10–50 cm of sinking and, without exception, reaching the bottom in this position.

Flagellates

It was not the aim of these studies to experiment with flagellar phytoplankton organisms because they are motile and this is probably a more effective tool for remaining in suspension than to evolve according to sinking resistance. Experimental data have shown that flagellates with a simple shape (*Rhodomonas, Gymnodinium*; Table 1; Plate II: b,c) had $\Phi < 1$ except *Ceratium* (Plate II: a) which fell into the range of other species with protuberances (*Staurastrum*).

Discussion

Rod-like forms are common in several divisions of phytoplankton. Typical representatives are oscillatorean and nostocalean cyanoprokaryota, *Aulacoseira, Planktonema, Mougeotia.* Evolutionary advantage of filamentous shape lays in the fact that they are good light antennae and therefore can photosynthetize with high capacity at low ambient light (Reynolds, 1997). An additional benefit of this shape is the rather strong increase of Φ with increasing length as was also found by Reynolds (1984) in experiments with killed *Aulacoseira italica* filaments with different cell numbers although Φ values in Reynolds' (1984) experiments were considerably higher (2.3–5.1) then in our studies.

If the relationship between Φ and L/D (Fig. 2A, $y = 0.053x + 1.1355$) remains linear at higher L/D ratios (we could not experiment with really long cylinders because size of our aquarium did not allow), for a filament having a diameter of 3 μm and a length of 300 μm Φ is expected as about 6.5 which is quite a high one as compared to others obtained in this study and agrees with Reynolds' (1984) highest data.

Indeed, there might be cases when high form resistance of long filaments significantly contributes to establishment of abundant populations in the upper section of a stratified water column. The best example is the establishment of an '**M**' assemblage (Reynolds et al. 2002) in the eplilimnia in summers like that dominated by *Planctonema lauterbornii* in the Vouglans reservoir, France (Leitão et al., 2003), occurrence of *Mougeotia* in Lake Garda, Italy (Salmaso, 2003), or, as most striking case, the annually recurrent under-ice development of *Aulacoseira baikalensis* in Lake Baikal, Russia (Kozhov, 1963).

As mentioned earlier, starting position of cylinders affected the sinking velocity of the particle. We consider this observation as consequential on experimental conditions only and rather irrelevant in nature (at least for non-motile plankton, see later). If starting our longest (8 cm) cylinder in vertical position, the sinking trajectory (approx. 80 cm) that the aquarium allows is only 10× longer than the filament itself. Moreover, the medium was static. The sinking trajectory of a 80-μm long filament within a 8-m thick epilimnion is 100 000 times longer than the filament itself and the medium is far of being still (convectional currents, wind-induced turbulences, etc.). Therefore, we assume that it will change its sinking position frequently thus averaging the differences that arise from different 'starting' positions if the term 'starting position' can be used for natural situation at all.

Disk-like organisms or rather isodiametrical cylinders with L/D < 2 (representatives: most species of Centrales) had a rather uniform Φ, therefore slight species-specific differences in their Φ probably do not contribute significantly to selection of a particular species in a given lake.

Colony formation of phytoplankton is usually viewed as a major tool for maintenance high physiological activity rates (since it depends on cell size) and at the same time avoiding grazing (since it depends on colony size). Both species tested in these experiments are common and very successful ones, moreover, they are among the few species for which earlier Φ records are published. For *Asterionella*, our results are in perfect agreement with Reynolds' (1984) findings in two respects: (i) Φ values in his experiments ranged from 2.5 to 4.3 for 1–16 celled colonies while our range was 1.6–4.9 for 1–14 celled colonies, and (ii) he also found almost an linear increase of Φ for 1–6 celled colonies and then Φ remained practically constant. Actually, the latter observation was the most convincing in respect that the method used in this study is an appropriate tool for studying sinking properties, despite the fact that the dimensions applied here were considerably magnified. In the case of *Fragilaria crotonensis*, Φ ranges also agreed with other published data (2.9–6 for 1–20 celled chains in Reynolds [1994] and 4.4–8.1 for 1–14 celled chains in our experiment); increase of Φ with cell number of the chain was close to linear, and, unlike *Asterionella*, apparently did not have an upper limit.

It should be mentioned that losing form resistance by fragmentation of the colony is not necessarily disadvantageous for the population. Fast growing populations of *Asterionella* consist typically of colonies with 8 or 16 cells, which have the highest Φ that this species can attain. If population decline starts – for whatever reason (parasites, depletion of nutrients, etc.) – colonies usually split into 1–3 celled fragments that sink in to the hypolimnion where the causes of population decrease do not prevail. There, they can perrenate and give a start for a new development, if and when environmental conditions allow. This recurrent behavior of *Asterionella* populations is well known.

There is quite a number of species or closely related species that have straight and coiled morphotypes. Examples include *Planktolyngbya limnetica* (straight) – *P. contorta* (coiled); straight and spiraling morphotypes of *Cylindrospermopsis raciborskii* (Fabbro et al., 1996; Baker, 1996); *Pseudanabaena limnetica* (straight) – *P. contorta* (coiled; Kling & Watson, 2003); *Gloeotila pelagica* (straight) – *G. spiralis* (coiled; Schmidt, 1994); *Aulacoseira granulata* var. *angustissima* and *Aulacoseira granulata* var. *angustissima* Morphotype *curvata*. The sinking properties of coiled forms have shown that coiling substantially decreases form resistance factors and therefore floating properties of straight or close-to-straight morphotypes are more advantageous. In grazing experiments carried out by Fabbro & Duivevdoorden (1996), *Brachionus rubescens* could ingest straight filaments of *Cylindrospermopsis raciborskii* while grazing on coiled filaments of *C. raciborskii* was not observed. Little is known about ecological advantages or disadvantages of coiling, however, it seems apparent that coiling decreases form resistance but increases resistance to grazing.

Experiments with star-like forms (*Asterionella*) and coenobia with spines (*Tetrastrum* colonies) have shown that symmetry of a cell or of group of cells have a high impact on Φ: symmetric forms have a higher form resistance than asymmetric ones and the relationship seems to be linear or close to linear. However, as found in the case of *Pediastrum*, certain kinds of symmetry features (arrangement of holes) do not affect the hydrodynamic properties of a sinking particle. Indeed, non-motile planktonic species in nature are frequently symmetric in both the arrangement of cells (if they are colonial) and in the external structures (like spines) on their surfaces. This symmetry in itself can be viewed as a tool to maximize resistance to sinking.

Protuberances and spines are rather common in phytoplankton groups Xanthophyceae, Chrysophyceae, Bacillariophyceae, Chlorococcales and Desmidiales. Such structures increase cell dimensions significantly without being too expensive in terms of cell energetic investment. For this reason, they are acknowledged defence mechanisms against zooplankton grazing. This early assumption got a solid support by experimental observations that spine development in some chlorococcalean algae can be triggered by infochemicals released by zooplankton (e.g. Schlütter et al., 1987), thus being a clear antigrazing property. Additionally (in some groups probably exclusively), spines are an effective tool to increase form resistance in case they are in symmetric arrangement on the cell/coenobium surface. Prior to this study, Conway & Trainor (1972) showed that *Scenedesmus* strains with spines sank more slowly than others without spines. Smayda & Boleyn (1966) found that spineless pre-auxospore cells of the marine diatom *Rhizosolenia setigera* sank faster than those with spines. In experiments when spines of *Thalassiosira weissflogii* were removed with chitinase (Walsby & Xypolyta, 1977): treated cells sank twice as fast as untreated ones. These early findings resulted in a Φ of no higher then 2. From our experiments, it can be extrapolated that very spiny cells/coenobia like *Micractinium*, *Chrysosphaerella* and most species of Heliozoa may have a Φ up to 4 and therefore spines may significantly contribute to keeping the cell in suspension.

Planktonic desmids that were studied in this study had rather low form resistance. Even such a complicated form as *Staurastrum rotula* with its alternating 12 arms had a Φ of some 1.5. Nevertheless, we found a significant correlation between arm length relative to cell width and Φ. We suppose that the suspension of planktonic *Staurastrum* species in the summer epilimnia is not due simply to the form resistance of the cells but to their hydrodynamic behavior in a kinetic medium. This deduction is supported indirectly by observations in stratified tropical and temperate lakes: in stratified temperate lakes, desmids often contribute an insignificant portion of total biomass. In tropical lakes, however, they can dominate in the epilimnion. This observation seems to be contradictory to the implication of the Stokes equation: sinking of a particle of given density and form resistance should sink faster in a tropical epilimnion than in a temperate one, simply because of the lower density of the medium. In order to understand their dominance in some tropical lakes, we have to compare the hydrodynamic properties of lakes at different latitudes. Owing to the wide extremes of day-night temperature variations in the tropics, either the whole water column (atelomixis) or at least the epilimnion (partial atelomixis) is subjected to daily overturn (Barbosa & Padisák, 2003). Thus, particles that manage to remain in the epilimnion in the stagnating diurnal phase of the day experience a robust redistribution during the hours of darkness. Night-time convection is generally much weaker in temperate latitudes, so particles with insufficient resistance to sinking fail to remain in the epilimnion during calm periods.

As was hypothetized in earlier works (McNown & Malaika, 1950), teardrop-shaped forms, like *Rhodomonas* and *Gymnodinium*, have a $\Phi < 1$. Reynolds' (1984) contention that small projections or irregularities of the cell shape or on the cell surfaces do not greatly reduce sinking velocity was clearly upheld in this study. For this reason, we do not suppose that other forms common among the Cryptophyta and Dinophyta would have markedly different Φ. There is an exception to this: *Ceratium* which a very successful genus both in freshwater and sea. It is a powerful swimmer; it is generally too big to be grazed; it has a clear diurnal migration pattern to avoid photoinhibition yet still harvest sufficient light energy to support net photosynthetic gain; and it can migrate to nutrient-rich deeper layers to suffice its nutritional demands if the epilimnion is seriously nutrient depleted (Heaney & Talling, 1980). As shown here, Φ in *Ceratium* 2–3 times greater than other flagellates. We suppose that the energetic cost to *Ceratium* of remaining in the required vertical layer is lower than that to other flagellates and this may contribute to success of this genus.

Although no chrysoflagellate was tested in these studies, it is tempting to extrapolate from this study to other algal groups. Forms of individual species in Euglenophyta, Dinophyta, Volvocales and Chrysophyta are quite similar in the context of their probable form resistance. Species of these groups Euglenophyta, Dinophyta, Volvocales are powerful swimmers (Sommer, 1988). Chrysophytes are not successful swimmers. Most unicellular chrysophytes inhabiting the epilimnia of stratified lakes are too small to be efficient swimmers (Sommer, 1988). Colonies comprising many flagellated cells (like *Uroglena*) cannot reach high unidirectional speed because flagellar movement of individual cells in the colony is not synchronized (Sandgren, 1988), as is the case in the superficially similar colonies of *Volvox*. In this way, it seems quite likely that the high morphological variability that is observed among the chrysoflagellates (like spines of *Bitrichia* or *Stephanoporos*, and silica spines in the genera *Mallomonas, Chrysosphaerella, Spiniferomonas*) were evolved to help their otherwise handicapped flotation.

When discussing form resistance of phytoplankton species, it is difficult to avoid mentioning the so called "vital factor". It has been demonstrated by a number of workers (Smayda & Boleyn, 1965; Eppley et al., 1967; Smayda, 1970, 1974; Reynolds, 1973; Titman & Kilham, 1976; Wiseman & Reynolds, 1981) that dead or even living but senescent algae sink faster than viable cells, by factors of three to five, but without any visible

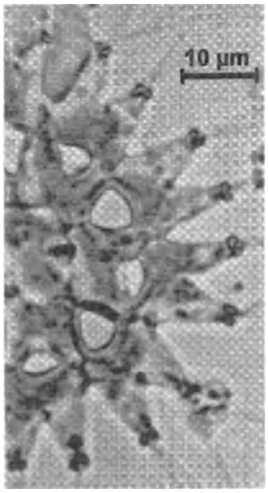

Figure 6. Pediastrum duplex from River Elbe. Note the mucilageus protuberances emerging on the marginal cells. (Photo: L. Krienitz. Published with allowance by L. Krienitz).

alteration in shape, size or form-resistant structures. Since this vital factor remains mysterious, Reynolds (1984) concluded that it is advantageous to separate its contribution to Φ by investigating form resistance in killed cells. This advice was wholly followed in this study, insofar as there is no vital contribution to the sinking behaviour of PVC. However, it is opportune to speculate what this vital factor might be.

Phytoplankton ecologists routinely work with Lugol-preserved samples and recent taxonomists are mostly busy investigating 16S rDNA sequences of cultured algae. One of the consequences is that many, seemingly unimportant, structures remain hidden, although they were quite evident for old-time taxonomists insistent on working with freshly collected living material. These structures include, for example, mucilage stalks or spine-like protuberances often surrounding the cells. On a photo published by Canter-Lund & Lund (1995; Fig. 37) a *Pediastrum* coenobium is seen with hardly visible mucilage stalks initiating on marginal cells. These structures are much more apparent on the photograph (Fig. 6) of a *Pediastrum duplex* from River Elbe: the specimen had numerous such structures originating from the marginal cells and radiating outwards. According to personal communication of the original observer, Dr Lothar Krienitz (see also Krienitz, 1990), these mucilage protuberances had lengths corresponding roughly to the diameter of the coenobium and the photo was taken on a freshly collected coenobium which was dried in under the microscope. Also in a dry preparation documented in Canter-Lund & Lund (1995, Fig. 214) a *Cyclotella* cell is seen with 10–15 times longer non-siliceous protuberances than the diameter of cell. Drying of freshly collected specimens seem to be an effective tool to make visible protuberances of algal cells and staining can be the other one: Canter-Lund & Lund (1995; Fig. 60) published the photograph of a *Staurastrum* stained with brilliant crystal blue and showing fine protuberances most probably made of mucilage stalks.

They might easily disintegrate when the cell is killed or even when they are merely senescent. We never see these structures in preserved materials even if methylene-blue staining or Indian-ink contrasting is applied. Needless to say that presence of such structures may significantly increase Φ and it is also probable that such flexible soft structures do not prevent grazing and therefore did not evolve for predation defense tool.

Another important kind of vital contribution to form resistance might be the ability of the living organism to maintain the position that provides the highest form resistance. As shown above, our *Tetrastrum* forms with many spines on only one side of the coenobium turned to 'spines up' position very rapidly and in this position they sank faster than the equivalent forms lacking spines altogether. If species with such asymmetrically arranged protuberances are motile, a main role of its motility can be to maintain a certain orientation of the cell in which ensures maximum form resistance. In phytoplankton, such species might include *Mallomonas tonsurata, M. corymbosa* and *M. cyathellata*. We cannot see position and swimming behavior in living, motile *Mallomonas* but we can see that of jellyfish, one of the biggest members of marine plankton. Medusae have jelly arms varying in numbers and size and they are invariantly below the main body of the living animal. We may even observe how they use their jelly arms for flotation between two active movement events. Note: this position of the arms is just the opposite from that we observed in asymmetric artificial *Tetrastrum* shapes. If a jellyfish is dead, it rolls in the water: the animal is visibly drifting in a rather random position and the jelly arms or their visible rests are never as beautifully arranged and symmetric as when they are living. It may follow that positioning of the cell, even in phytoplankton, is an essential vital component of sinking especially for non-symmetric planktonic plants or animals with at least some motility.

Form resistance is of course not the only adaptive mechanism for enhancing flotation of plankton. Planktonic animals and most flagellate phytoplankton species are quite efficient in vertical positioning. Cyanoprokaryota have very effective mechanisms for regulating the buoyancy provided by their gas vesicles or aerotopes. Mucilage seems to be a flotation-aid however the mechanisms are unclear since on one hand it decreases the density of the organism but, on the other hand, increases its size. However, on basis of the Stokes' equation, we have to see that decrease of density, for example by mucilage secretion, is an ultimate tool for remaining in suspension: an organism with density equaling the density of the medium will float independently of size or shape.

It is difficult to assess how much mere form resistance has contributed to the evolution of planktonic species or to the selection of specific phytoplankton assemblages. Nevertheless, differences in form resistance can be decisive in some cases and it can also be supposed that at least part of the overall morphological diversity of plankton developed under evolutionary driving force of form resistance optimisation.

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Aquatic biodiversity and saline lakes: Lake Bogoria National Reserve, Kenya

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Key words: African Rift Valley, spirulina *Arthrospira fusiformis*, lesser flamingo *Phoeniconaias minor*, Ramsar, avian tuberculosis

Abstract

Lake Bogoria, in the Rift Valley of Kenya is an extreme saline lake (conductivity 40–80 mS cm^{-1}, alkalinity 1500 m equ l^{-1}). It is hydrologically more stable than the other, endorheic lakes in Kenya, because it is deep – maximum depth at present just over 10 m in an area of 3000 ha – and so does not have periods when it is dry. It is ecologically simple, with only one species dominating the phytoplankton – the cyanobacterium 'spirulina', *Arthrospira fusiformis.* Its biomass and productivity were very high – biomass between 38 and 365 $\mu g\ l^{-1}$ chlorophyll '*a*' and 3.4–21 $\times 10^3$ coils ml^{-1} and net production between 0.24 and 1 gm C m^3 h, the latter in a narrow zone of less than a metre. There were no macro-zooplankton in the plankton and the only grazer of *A. fusiformis* was the lesser flamingo, *Phoeniconaias minor,* which occurred irregularly in very high concentrations (in excess of 1×10^6). Detritivory in the benthos was effected by a single chironomid species, *Paratendipes* sp., at a maximum density of $4 \times 10^4\ m^{-2}$. The mean daily emergence of adult chironomids was estimated to be $1 \times 10^3\ m^{-2}$, the maximum 3. There was no littoral plant community within the lake but 44 dicotyledonous and 31 monocotyledonous plant species in the drawn-down zone and adjacent to it. A diverse draw-down terrestrial invertebrate fauna, only superficially described here, processed the flamingo feathers and carcasses, with other detritus such as chironomid pupal exuviae and decaying *A. fusiformis* scum. About 50 bird species depended upon the chironomids, either as they emerged through the water column as flying adults or later on the shoreline as floating pupal exuvia and dead adults. The lake has high conservation value because of three bird species in particular – lesser flamingo, Cape teal and black-necked grebe. The former provides real economic value in a region otherwise impoverished, because of the spectacle of tens of thousands of flamingos set against the landscape of hot springs and fumaroles at the lake edge, which draws 15 000 visitors per annum. *P. minor* has experienced three periods during the past ten years when major mortalities have occurred, the last of which killed 700 birds day^{-1}. This could have involved as many as 200 000 birds (about 1/5th of the maximum population at this lake) if mortality was at a constant rate for the nine months it was observed. Causes of mortality have been suggested as avian tuberculosis, poisoning from cyanobacterial toxins or from heavy metal contamination at Lake Nakuru, but it is still not yet clear what contribution each makes to the problem.

Introduction

Biodiversity is a word with a short life history, even though Henri Dumont has been a practitioner of it for his long and productive professional life. Probably first used in 1984 at a conference in Washington DC, U.S.A. (Wilcox, 1984) and several times in quick succession thereafter (Wilson, 1987, 1988), the number of times it was used in the literature accelerated through the early 1990s (Harper & Hawksworth, 1994). Its entry into everyday English is a result of its widespread use at the 1992 Rio de Janeiro Conference on Environment & Development (popularly called the Earth Summit). This UN Conference helped to focus global attention on the rates of loss of biodiversity – extinction of species and populations – that are now considered so alarmingly high by many scientists as to threaten the life-support systems of our planet (Wilson, 2002). At best, deterioration of these life-support systems will be financially crippling (Constanza et al., 1997), at worst it will reduce the capacity of the planet to sustain humans (e.g. water resources; Vosmarty et al., 2000).

Henri's life has been devoted to improving our knowledge of aquatic biodiversity and limnology. On the one hand he has made considerable advances in taxonomy, particularly of crustacea, and on the other hand he has advanced our understanding of aquatic ecosystems and their processes through his development of *Hydrobiologia*. An aquatic ecosystem promoted extensively in this journal has been the saline lake, particularly through the inclusion of the edited conference proceedings of six scientific meetings devoted to it (e.g. Melack et al., 2001). Saline lakes present several interesting facets of aquatic biodiversity and Lake Bogoria, Kenya, illustrates these *par excellence*. There has been no previous publication specifically on this lake's limnology or biodiversity and it is appropriate therefore, that the first one appears in this volume.

Much of the knowledge of saline lakes in Africa has come from studies of their chemistry (e.g. Talling & Talling, 1965; Wood & Talling, 1988), biodiversity (e.g. Jones et al., 1994) and the magnitude of their primary production (e.g. Melack & Kilham, 1974; Melack, 1981). It is well understood that saline lakes have a limited species complement in macro-organisms in contrast to considerable biodiversity in micro-organisms (Beadle, 1981; Grant et al., 1990; Duckworth et al., 1996) and that production of the few species that dominate each trophic level is high (Vareschi, 1987). Attention has now focussed on their temporal dynamics (Verschuren et al., 1999), because of the fundamental scientific interest but increasingly because of the applied value in understanding human interference with their hydrological balance [e.g. Mono lake, California (Jellison et al., 2001) and Lake Nakuru, Kenya (Anon, 2003)]. In African saline lakes, this applied value has a unique facet because these lakes have economic value to the local human population through tourism based almost entirely on one species – the lesser flamingo (*Phoeniconaias minor* Geoffroy).

P. minor is the major primary consumer in East African saline lakes in particular, but also in South Africa and parts of West Africa (Simmons, 2000), filtering the planktonic cyanobacterium 'spirulina' (Ridley et al., 1955), *Arthrospira fusiformis* (Voronichin) Komárek (Hindák, 1985) together with shallow littoral diatoms in lakes and a wider range of cyanobacteria and diatoms in temporary wetlands (McCullough et al., 2003) These flocks of *P. minor* give the term 'biodiversity value' an unusual slant, because it is elsewhere generally synonymous with species diversity. Lake Nakuru, the first protected area in Africa for birds alone (and Kenya's first Ramsar site), was primarily purchased and conserved for this one species; is the second most visited National Park in Kenya and was considered "the most fabulous bird spectacle in the world" by Roger Tory Petersen (Williams, 1967). Lakes Bogoria and Elmenteita are the other two Kenyan saline lakes used extensively by *P. minor* (Owino et al., 2001). The former is a protected National Reserve and Ramsar site; the latter privately owned but managed sympathetically. *P. minor* occurs in flocks of over a million individuals at these lakes (see below) but is considered to be a 'near-threatened' species (Hilton-Taylor, 2000), because the numbers are only about half of those formerly recorded [1.5 million Lake Nakuru (Vareschi, 1978), 2 million Lake Bogoria, (Brown, 1959)] and it only has one regular breeding site in East Africa and breeds there only intermittently – Lake Natron in Tanzania. Moreover, three large mortalities of *P. minor* have occurred in Kenya in the previous decade; both at lakes Bogoria and Nakuru in late 1993 and late 1995, at Lake Bogoria alone in late 1999. At least 40, 20 and 80×10^3 birds, respectively are estimated to have died in these three incidents (by subjective media reports at the time; no scientific numbers have been published). Two decades earlier, in late 1973 several hundred birds had died at Lake Nakuru, coincident with a sharp decline in the biomass of their food (Vareschi, 1978) and avian tuberculosis was identified in postmortems (Cooper et al., 1975; Sileo et al., 1979). The deaths during the 1990s, currently not convincingly explained, threaten *P. minor*'s economic value through depleting tourist income, as well as its conservation status.

P. minor is a nomadic species, moving between the lakes on which it feeds at irregular intervals in unpredictable numbers. Leslie Brown, the first biologist to study flamingos objectively in the 1950s and who discovered their breeding sites (Brown & Root, 1971) wrote "Personally, I hope that no one ever will fully rationalise flamingos, and that they will remain the supremely beautiful, elusive, opportunistic, unpredictable beings I like to think they are" (Brown, 1979). Their nomadic behaviour has probably evolved as a response to the unpredictable dynamics of their food supply in the short-term and the unpredictable status of the lakes in the long-term.

Most of the Rift Valley lakes have shown major hydrological changes on a time scale of centuries and longer, their limnology alternating between saline and fresh, from the paleolimnological evidence in sediment cores (e.g. Lake Abiyata, Ethiopia; Legesse et al., 2002; Chalié & Gasse, 2002). Lakes Elmenteita and Nakuru were once joined, forming a single large freshwater lake (Nilsson, 1932) but are currently separate, shallow and saline. Nakuru has dried up 7 times in the past 70 years (Vareschi, 1978; Nasirwa, 2000) and Elmenteita also at least once per decade (I. Marshall, pers. comm.). Both lakes experienced an order of magnitude change in their alkalinities over 8 years in the 1960s, between 122 and 1440 meq l^{-1} (Livingstone & Melack, 1984). Bogoria was also once part of a larger freshwater lake, joined with the current Baringo (Nilsson, 1932) but by contrast with the other two, it is currently still moderately deep, with a maximum depth recorded as 12 m (Vareschi, 1978). It is thus likely to be both physically and chemically more stable than the other two in drought conditions.

Lakes Nakuru and Elmenteita were intensively studied during the 1970s for several consecutive years (Vareschi, 1982; Melack, 1988). These authors recorded one longer period of stable, high photosynthesis and biomass in the early seventies and one short period of unstable, low photosynthesis and biomass in 1974. It was hypothesised then, that the changes were triggered by the rate of increase in salinity as water level declined in drought conditions (Melack, 1988). This decrease in primary producer biomass also resulted in decreases in *P. minor* at these lakes (Vareschi, 1978; Tuite, 2000). Lake Bogoria was sampled less often during this period of study in the 1970s (see below) and so the extent to which Bogoria is more stable than the two other lakes remains unclear.

The objectives of this study were to establish whether the chemical and biological parameters of Lake Bogoria are more stable than those of the two shallow lakes and what the consequences of chemical changes might be for lake ecology and biodiversity, particularly flamingo behaviour and biology.

Some preliminary measurements (not hitherto published) were recorded in the open water in the 1970s; an investigation of the microbial flora of lake water was undertaken over one year in 1988–9 and then five visits were made between February 2000 and February 2003 in which both limnology and biodiversity were investigated. This paper describes the lake's limnology and its links to the National Reserve's biodiversity, from these three periods. It forms a prelude to a longer multidisciplinary project, funded by the U.K. Darwin Initiative (http://www.darwin.gov.uk/projects.htm) which will make monthly visits from July 2003–June 2006.

Study site

The Eastern (Gregory) Rift Valley of Africa contains several dozen lakes ranging in size from Lake Turkana, 250 km long, in northern Kenya to many small (<1 km) volcanic crater lakes. All of them are endorheic in depressions on the valley floor or inside volcanic craters. Most of them, as a consequence, are saline to a greater or lesser degree (Talling & Talling, 1965). Lake Bogoria lies in a trough, created by tilt faulting which is characteristic of this area of the Rift in northern Kenya (Fig. 1). It is 16 km long and 1–4 km wide, on a north-south axis with three basins between two 'necks' formed by raised ground.

The rainfall pattern of this area is complicated by the topography of the Rift here, which widens in a triangular shape northwards with Bogoria close to the southern apex. Precipitation is influenced by both the Rift's escarpments and by mountain ranges rising from the widened Rift floor. The lake itself lies in a relatively low altitude (975 m), low rainfall area (mean 708 mm 1976–2001) and experiences two rainy periods associated with the Inter Tropical Convergence Zone in April–May and October–November plus an additional July–August peak attributed to a westerly

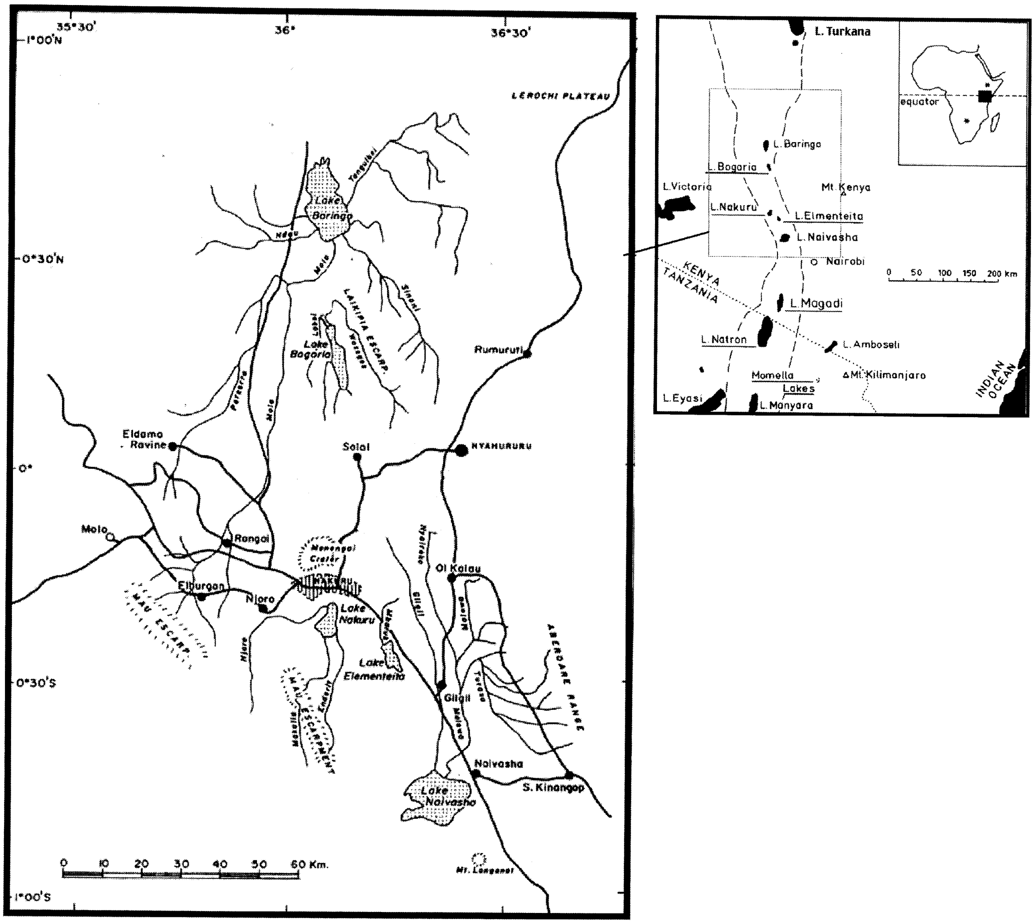

Figure 1. Saline lakes (names underlined) within the lake series in the floor of the Gregory Rift Valley of Kenya. Modified from Vareschi (1978).

air stream (Davies et al., 1985; LaVigne & Ashley, 2001). The hydrology of the lake is complicated by the inflow from numerous hot springs along its western and south-eastern shoreline in addition to its 5 river inflows. Three of these are permanent; two small freshwater streams, fed by springs, enter in the south while one river, the Ndolaita-Loboi, which formerly flowed northwards, has been diverted for irrigation and flows in the northern end of the lake. Two seasonal rivers, with substantial deltas, enter the north and west (Fig. 2).

The chemistry of the lake was initially studied by Jenkin (1936), Talling & Talling (1965), Vareschi (1978) and Melack (1981). The latter two papers recorded that the lake had a temperature range of 24–31°C, conductivity of 72–77 mS cm^{-1}, alkalinity 1500 meq l^{-1}, and pH of 10.2–10.3 in 1974. Its general alkaline state is a result of strong equatorial evaporative concentration, which leaves a sodium- and carbonate- dominated solution (Livingstone & Melack, 1984; Grant et al, 1990).

Methods

The lake outline was taken from the 1:50 000 maps of the Survey of Kenya, 1973, which were based on aerial photographs flown in January–February 1969. Bathymetry was measured from an inflatable dinghy during the period 16–26th August 2002 using a Lowrance X-15A chart recording echo-sounder with a 20° transducer beam. Positions of key depth readings were located with a Garmin 12 hand-held GPS receiver, enabling contours and maximum depths to be mapped.

Water samples from 1972–78 and 2000–3 were collected in replicate from a dinghy in open water stations over the deepest point in each of the three basins; in 1988–9 from the western shore at two locations in 30 cm of water depth on 12 consecutive monthly occasions. Water samples were collected at predetermined depth intervals from Rutner bottle (1972–8) and either from a rubber tube and pump (from the upper half metre) (1972–78) or a plastic Van Dorn-type messenger-closed 1.25 l sampling bottle (from 1 m and below) (2000–3). Secchi disc transparency was measured using the extinction of a 20-cm diameter black and white quartered disc. Conductivity and temperature were measured using a WTW conductivity meter with Withney electronic thermometer (1972–8), Markson portable conductivity meter with automatic temperature correction (1988–9), YSI (Yellow Springs Electrode company) model 50 Conductivity meter (2000–2) and YSI 6600 Multiparameter Sonde (2003). pH was measured on WTH, Pye-Unicam Gingold, Hach and YSI instruments. Oxygen was measured by Winkler titration (1972–8) and YSI model 58 meter (2000–3). Alkalinity was titrated with 0.1N HCl using phenolphphalein and Bromocresol Green-Methyl Red indicators.

Viable counts of bacteria on the lake water collected in 1988–9 were made by Colony Forming Units (CFU) cultured on Horokishi medium modified by addition of 4% NaCl, for 48 h at 37 °C. Total counts were made using epifluorescence microscopy on a water sample with acridine orange stain, filtered through black Millipore filter (0.22 μ pore size). Immersion oil was placed on the filter paper and bacteria counted at 1000 × magnification on a Leitz microscope with blue epifluorescence light.

A. fusiformis was counted microscopically, a minimum of 4 times on each water sample, by mounting 1 ml of shaken sample in a plastic Sedgwick Rafter cell and then counting 20 squares at random under 40 or 100× magnification on a compound microscope. Numbers of colonies of *A. fusiformis* were counted and then number of coils calculated from a single determination of the mean number of coils per colony for each visit. Other phytoplankter species were searched for using a microscope at 100 and 400× magnification from a composite sample.

A fixed volume of each water sample was filtered through Whatman GFC filter paper to retain the phytoplankton. The papers were placed in the dark until all samples had been filtered for chlorophyll '*a*' determination. When filtering was complete, the papers were each cut into strips and then ground in a pestle and mortar in a small quantity of 90% alkaline acetone and a pinch of sand for 5 minutes. The contents were carefully washed using more acetone into a 15 ml centrifuge tube after no green flecks could be seen, the tube made up to the mark and then centrifuged for 5 min at maximum speed on a hand-operated centrifuge. The liquid was carefully decanted into matched glass tubes and read against acetone blank in a Hach DR 2000 portable spectrophotometer at 750 and 665 nm. Phytoplankton chlorophyll '*a*' pigment concentration was then determined using the approximate equation of Talling & Driver (1963).

Primary production was measured in water collected from 15 to 25 cm, enclosed in Winkler (1972–8) or 250 ml 'Pyrex' glass reagent bottles (2003) which were suspended at the same point in the lake in pairs horizontally and pairs in light-proof containers vertically, at fixed depths for a measured time. Oxygen concentrations were measured at beginning and end by Winkler determination (1972–8) or YSI Model 58 Oxygen meter with pre-calibrated BOD probe immersed in the bottles and mechanically-agitated (2003).

Benthic samples were taken from measured depth with an Ekman grab of 15 × 15 cm and the mud filtered through a 25 micron mesh sieve over the side of the boat. Invertebrates were sorted live and counted in the laboratory, then blotted with damp tissue paper and wet-weighed, before preservation in 70% alcohol.

Flowering plant species growing within the draw-down zone and within 100 m of the current lake edge were identified from Agnew & Agnew (1994), grasses from Weiss (1989), and sedges and rushes from Haines & Lye (1983). Coleoptera inhabiting this zone and the riparian *Acacia* scrub were sampled by beating and sweeping using a standard entomologist's sweep net and by hand searching under the stones, dead wood and litter. A Heath portable moth trap in conjunction with a white suspended sheet was used for flying individuals. A series of pitfall traps was also used to sample the ground active coleoptera on vertical transects from the water's edge into the draw-down zone.

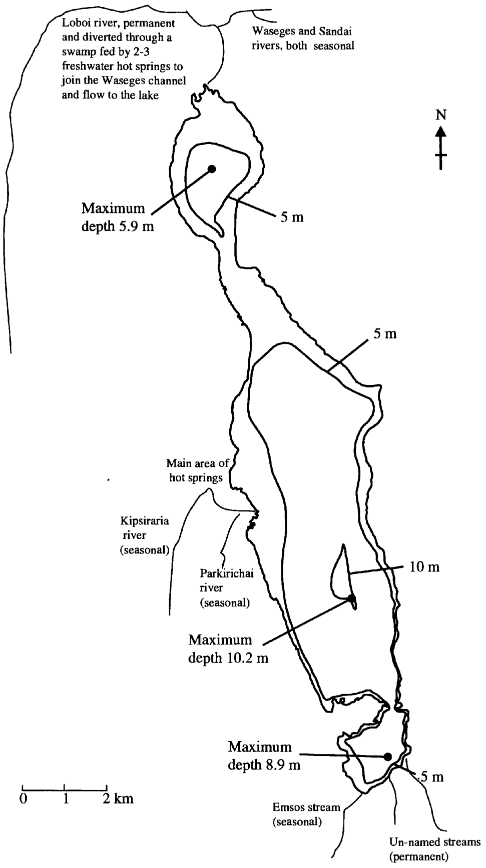

Figure 2. Details of Lake Bogoria showing inflows and bathymetry at August 2002 with 5 m depth contours and positions at which maximum depth was recorded in the three basins.

Bird species were identified using 10 × 42 binoculars and the Field Guide of Stephen & Fanshawe (2002) at all times during the visits 2000–3. Lesser flamingos on the lake were counted on each visit. Two–5 observers drove along the Reserve road from the south-west corner to the north-west. The vehicle stopped at every vantage point to divide the shore up into recognisable small sections, with one or more 'flocks' of birds. Each observer then recorded the numbers by counting 100 and estimating the number of 100-bird subgroups in the flock, using binoculars (10 × 42) or telescope (20–60 × zoom) to view the eastern shore. The observers worked independently; if their numbers were <5% of each other an average of the group was taken; if it was greater the count was repeated by all. At the end of the day the numbers in the sections were summed.

In February and December 2000, and August 2001, the mortality of *P. minor* was calculated from daily censuses at dawn of 5, 1-km lengths along the north and western shoreline in each basin over 12 consecutive days. Dead birds were removed from the shoreline before the start of the exercise and each day as they were counted. On one day, in the middle of the counting period, in 2000, the eastern shores were walked in their entirety in order to quantify the carcasses that were there. On other dates in 2002 and 2003 mortality was estimated.

Results

The majority of the lake is deeper than 5 m (Fig. 2) with shallower areas associated with deposition from the inflowing rivers in the north and west and along the neck of the lake between the central and northern basins. The deepest point was 10.2 m in the central basin. Lake surface elevation is now regularly recorded at the southern shore of the lake (W. Kimosop, pers. comm.) and this shows that lake level decline has been about 2 m, continuous for the last 3 years, which accounts for the deeper maximum published for the 1970s by Vareschi (1978).

The main chemical parameters showed some variation between 1972 and 2003 (Table 1). The largest change was in conductivity, which was half the value in 1978 and in 1988/9 that it achieved in 1974 or 2000–2003. There are no figures of lake level available for this period, but the rainfall collected at the Reserve indicate that the two periods of low conductivity corresponded to high rainfall associated with 'El Niño' events (77–79 & 88–90). (The heavy rain commenced in April 1977; the conductivity was recorded in the first 2 months of that year). The limited range of parameters measured over this period nevertheless indicate that the lake water is well buffered against changes, with pH only changing between 0.7 units (bearing in mind each visit used different instruments) and alkalinity changing little between heavy rainfall periods (88–89) and drought periods (1974 and 2000–3).

Replicates taken either from the north and south basins in 1988–9 or from a boat in all three basins in 2002 and 2003, resulted in low variance, indicating spatial uniformity throughout the lake. In August 1978 however, the middle of the 'El Niño' event, clear

Table 1. Chemical parameters recorded at Bogoria from 1972 to 2003

	pH	Conductivity mS cm^{-1}	Alkalinity meq l^{-1}
1972–3 ($n = 4$)	10.0	52.3	
1974 ($n = 2$)*	10.25	74.5	1500
1975–7 ($n = 3$)	10.3	59.0	
1978 ($n = 2$)	10.1	44.0	
1988/9 ($n = 24$)	10.7$^{+}/_{-}$0.2	36.8 $^{+}/_{-}$11.7	1160$^{+}/_{-}$14.2
2000–3 ($n = 12$)	10.3 $^{+}/_{-}$0.25	74.2 $^{+}/_{-}$ 2.6	1190 ($n = 1$)

*From Vareshi (1978) and Melack (1981); one measurement published in each.

chemical stratification was recorded in the south basin only, with the upper 5 m showing 60% of the conductivity of the deeper layers (Fig. 3a), which was not mirrored by pH differences (between 10.1 and 10.2 at all depths).

Very clear vertical stratification of oxygen was recorded on every boat sampling occasion from 1972 to 2003. In the south basin (sheltered) the oxycline always occurred between 1 and 2 m (Fig. 3b), but in the north and central basin (more exposed to prevailing winds) it was replaced by a more gradual decline from super-saturation at the surface to anoxia below 5 m. The surface waters were always supersaturated with oxygen, up to to 300% during the daylight hours, with a daily maximum in the afternoon between 1500 and 1700 (Fig. 3c). The temperature, by contrast, varied relatively slightly. The water below 4 m was between 23 and 25 °C on every occasion the lake was studied between 1972 and 2003. Surface water was at the same temperature morning and evening, but rose as high as 32 °C in the upper metre (Fig 3d).

Biological characteristics indicated up to an order of magnitude variation between sampling occasions (Table 2), with biomass of chlorophyll '*a*' from 38 to 363 mg m^{-3}. The density of *A. fusiformis* and the chlorophyll biomass varied approximately five-fold between 2000 and 2003 (2.7–21 × 10^{3} coils ml^{-1} and 38–165 mg m^{-3} chlorophyll '*a*') with a progressive rise through the four years. The low frequency of visits may give a deceptive air of stability, because a lake-wide *A. fusiformis* crash occurred in October 2001 (Nasirwa, pers. obs.), with aroma from the decay detectable several kilometres from the lakeshore (Kimosop, pers. comm.), although it is not known what the consequences were for succession or primary production, and no adverse effects of *P. minor* occurred. In all years studied *A. fusiformis* was the overwhelmingly dominant phytoplankton species. It represented over 70% of the community sampled throughout 1988–9 when the sample was collected from the shallow littoral but 100% of the true plankton community in 1972–8 and 2000–3 with no other species entering the counts. Diatoms were occasionally seen in the plankton, but were most likely vagrants from the shallow mud and hot springs inflow streams, where 34 species have recently been recorded (Escuté-Gasulla et al., 2002) (see also Gasse, 1996). Other cyanobacteria were also common in the hot inflow streams.

Spatial differences in *A. fusiformis* density and biomass occurred between basins on any one day, of up to 2–3 times, but this was not great enough to show differences in transparency. Observations from the air and from lake-side vantage points, indicated that wind-generated slicks concentrated lines of *A. fusiformis*, which attracted feeding *P. minor*; this short-term spatial pattern will be the subject of another paper (Vareschi, Mills, pers. obs.). Vertical differences within slicks could however be extreme; in May 1972 biomass of *A. fusiformis* in the top 5 cm was 20 times greater than that in the upper 25 cm.

The primary production of phytoplankton was confined to the upper metre of water surface, in all years when measured – 1972, 1974 and 2003. Net production ranged between 0.8 and 3.3 gm 0^2 h^{-1}, or 0.2-1 gm C h^{-1}.

Six strains of mainly gram-negative alkaliphilic bacteria, such as *Vibrio* sp., were commonly recorded in the water of Lake Bogoria during the 12 months when its microbial community was studied, 1988-9. Three of them, which occurred every month, accounted for over half the density. Overall, the mean density expressed as CFU was 3.85 × 10^{5} ml^{-1}, two orders of magnitude lower than the total epifluorescence count of planktonic bacteria. The taxonomy of these alkaliphiles is a poorly-understood area still being investigated (W.D.Grant, pers comm.).

There were no zooplankton above the size of protozoa in the water column at any time and only a single chironomid species of zoobenthos grazer/detritivore. This species, tentatively identified as *Paratendipes* sp., occurred in high densities throughout the lake above the anoxic depths (Fig. 4), with no difference between lake basins or between sampling date, 2000–2003. Maximum larval densities at 1–2 m depth were 3.9 × 10^{4} m^{-2} (wet weight 65.3 g), with a mean for the lake

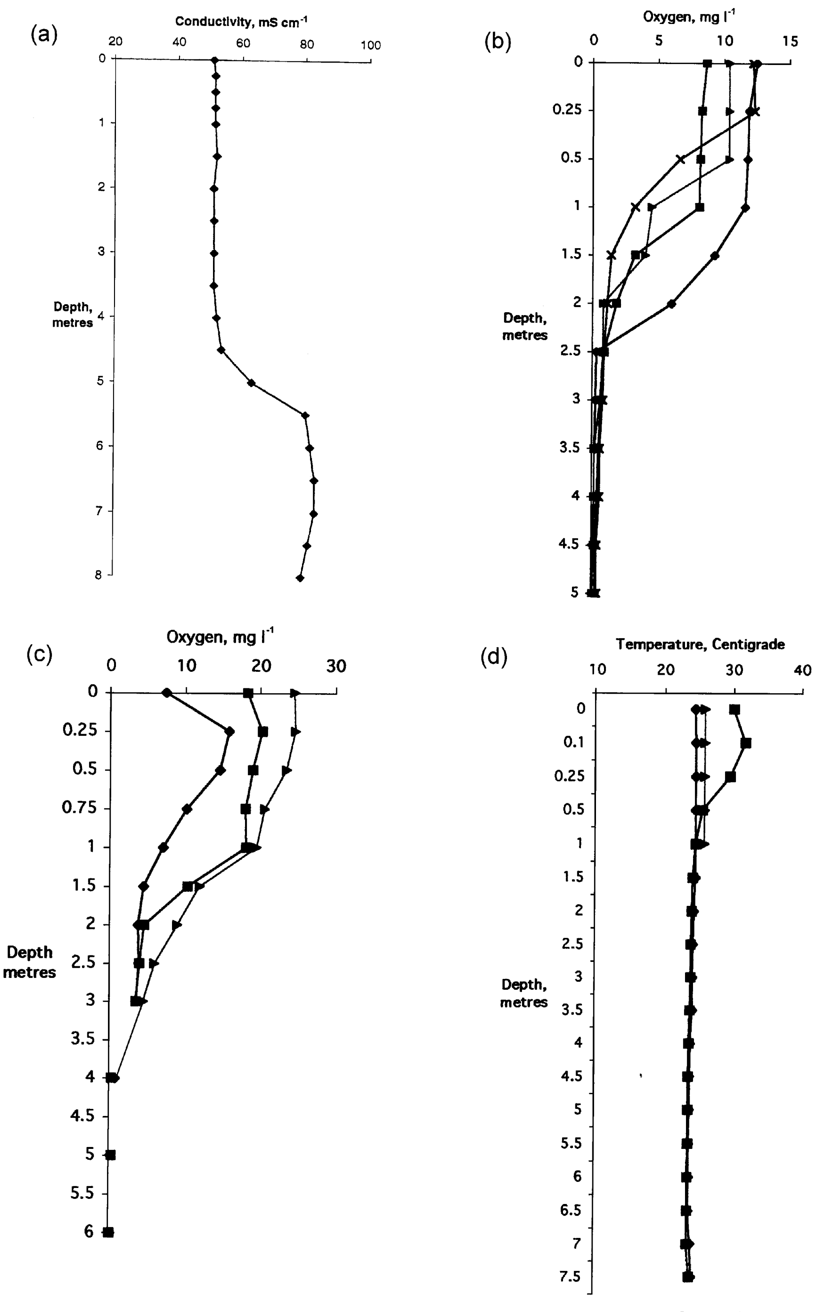

Figure 3. Vertical stratification of chemical parameters at Lake Bogoria, 1972–2003. (a) conductivity stratification in August 1978. (b) Oxygen stratification in February 2000 (diamonds), February 2001 (squares), October 2002 (triangles) and February 2003 (crosses). (c) Diurnal pattern of oxygen supersaturation in August 1978 between 1100 hrs (diamonds), 1500 hrs (square) and 1700 hrs (triangles). (d) Temperature stratification in February 1972 between 0800 hrs (diamonds), 1300 hrs (squares) and 1700 hrs (triangles).

Table 2. Biological parameters recorded at Bogoria from 1972 to 2003

Month/Year	1972	1974*	1978	1988–9	02/2000	2001[1]	10/2002	03/2003
Secchi disc cm, mean	–	36.5	–	–	53	23	19	18
standard deviation or range		28–45			5.7	3.7	2.3	1.1
n		2			9	18	22	18
Chlorophyll mg m^{-3}, mean	163	350	276	363.3	37.9	56.8	142.1	165.2
standard deviation or range	74	150–800	60	487.7	4.8	24.4	24.3	79.4
n	12	2	4	24	20	12	9	10
A. fusiformis coils ml^{-1}	2773	–		–	3375	11 639	14 520	20 826
standard deviation	750				1652	2152	1325	1151
n	15				23	12	11	15

*From Melack (1981).

[1] *A. fusiformis* counted March–June, but chlorophyll measured in March & August.

under 5 m of 1.4 × 10^4 m^{-2}, (wet weight 20.4 g). Adult emergence appeared to be continuous; with an estimated life cycle of 2 weeks (given the adult size and lake temperature), so the maximum daily production could be 3000 adults m^{-2}, mean 1000 m^{-2}. A conservative wet weight biomass is in the order of 3 tonnes, with a daily emergence of 210 kg, for the whole lake. This sustains several thousand individual avian predators; the most numerous are swifts and swallows and the most important for biodiversity conservation are the Cape teal and black-necked grebe (see below).

The density of *P. minor*, major grazer of the phytoplankton in the lake Bogoria ecosystem, varied rapidly, doubling or halving during the two and a half weeks of a sampling campaign, so the interpretation of numbers over a short time scale without substantial accompanying environmental data would be misleading. Its minimum numbers were 30 000 in February 2003, its maximum 510 000 in August 2001.

The most recent and the largest mortality of this species is believed to have commenced in July 1999 (Gough, 2000) and petered out in March 2000 (W. Kimosop, pers. comm.). The average daily mortality on the western side of the lake in late February 2000, was 28.8 $^{+}/-$ 5 individuals km^{-1}, with no evidence of either temporal (over 12 days) or spatial (between basin) differences. On the single count of south and eastern shores, only 25 flamingo carcasses, of all stages of decay, were recorded, which indicated that prevailing winds brought almost all carcasses to the western shore. Extrapolating the western shore counts to the length of shoreline exposed (west and north), gives a daily mortality for the lake of approximately 700 birds. This leads to a speculative mortality of 2 × 10^5 deceased birds for the nine months of the event, equivalent to about 20% of the late-1990s observed population (Owino et al., 2001). Comparison of this with mortality estimates subsequently made, in December 2000, August 2001, October 2002 and February 2003, suggest that smaller peaks of mortality occur on top of a base mortality (approximately 0.01% would be an average daily mortality of a bird with lifespan estimated at between 35 and 50 years). In December 2000–January 2001, when the number of *P. minor* went from 40 000 to 297 000 in 3 weeks, average mortality was measured as 9 $^{+}/-$ 4 birds day^{-1}. In August 2001, however, when between 510 000 and 325 000 birds were counted at the lake, 113.5 day^{-1} died. In October 2002 and February 2003, fewer birds were present – 92–94 × 10^3 and 40–20 × 10^3, respectively. Whole-lake mortality was estimated (not counted) as no higher than 10 per day, but at this low number it was difficult to distinguish between birds healthy but predated (by marabou stork, fish eagle or steppe eagle) and birds which had been close to death and were scavenged. Visual examination of the weak birds – between 20 and 50 – in a sub-population of circa 5000 on each of 12 days in February 2003, showed that less than 1 in 20 of weak birds was 'sick' (characteristic staggering and head-drooping): the remainder had visible damage to limbs (and occasionally wings) but held necks up, so were 'injured'.

In 2002 and 2003, over 50% of the population at the lake consisted of birds under 2 years old (white-grey plumage), which indicate that breeding had replaced the 2000 mortality. Breeding was still in evidence by March 2003, with an estimated 20 000

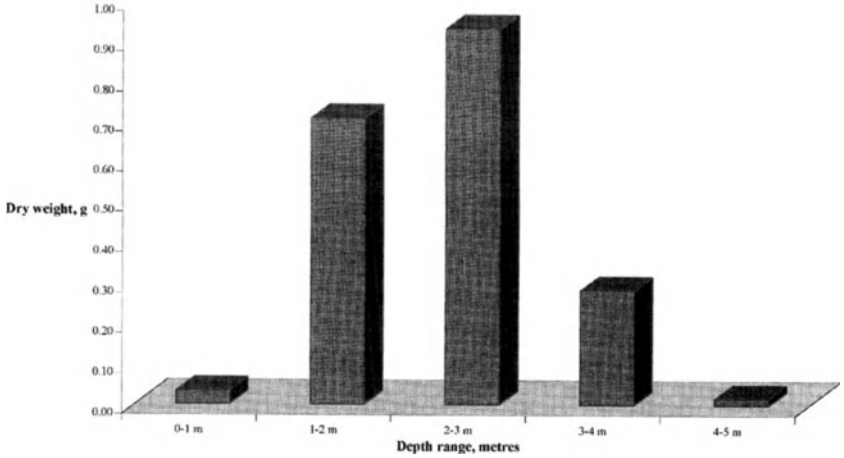

Figure 4. Mean distribution of wet weight of larvae of the midge *Paratendipes* sp. from 36 Ekman grab samples in February 2002. The standard deviations are not shown because the variances were very high (SD =/> mean) due to extensive density variation under 2 m depth.

chicks visible on Lake Natron (DMH, MMH, EV & A. Simpson, pers obs.).

No aquatic higher plants occurred in the lake. However, a diversity of plants have colonised the draw-down zone. Twenty-three families and 27 species of herbs and low-growing shrubs were recorded close to the shore (Table 3). Plant densities, although not necessarily species richness, were low in all areas other than around freshwater inflows. Adaptions to water stress, salt stress and removal by herbivores appeared to be more important in shaping the plant community than competitive interactions between individuals or closely related species. The high plant taxonomic diversity at family level is consistent with the wide range of habitats that are created by different substrate, salinity and water table depths and with a considerable breadth of plant adaptations to environmental stress. Selection appears restricted to the level of genus with typically only one or two species in each genus. The shoreline was rich in grass species, with 27 species. Of these, only *Sporobolus spicatus* and *Cyperus laevigatus* are true halophytes. The ratio of monocots to dicots in the ground flora was 1:1.3, which is much closer to the ratio expected in aquatic plant communities than the ratio of 1:4 more typical for terrestrial plants. Only three herbaceous species; the two lilies and the *Commelina* sp., are monocotyledons. The Liliiflorae are likely to have been under-represented, as bulbs and tuberous roots sometimes uncovered in dry and stony areas, had no evidence of aerial growth.

The water's edge consisted of a marginal 'drift' of flamingo feathers, remains of flamingo carcasses, bird droppings and vegetation debris, overlying rock-strewn gritty mud. This attracted high numbers of coleoptera, particularly tenebrionids, such as *Gonocephalum* sp., *Sepidium* spp. *Vietomorpha* spp., and *Rhytinota praelonga* Koch to nocturnally scavenge and shelter by day under stones. During the day the lake margin was dominated by *Zophosis* spp. and the predatory cicindelid (tiger beetle), *Lophyra boreodilatata* (Horn), commonly 'hawking' and running over the mud and bare sands. The lake edge scrub supported many cerambycids (longhorn beetles), including the large prionids, *Tithoes confinis* Castelnau and the ubiquitous *Macrotoma palmata* (Fabricius), which are primary agents in breaking down dead wood. Tiger beetles caught in light traps were *Prothyma methneri methneri* Horn, *Cylindera rectangularis* (Klug), *Myriochile vicina pseudovicina* (Mandl). In wetter periods Scarabaeidae dominated light trap catches, particularly rutelids and melolonthids. Elaterids (click beetles) were common, including the 6–7 cm long *Tetralobus* spp.

The vegetated riparian zones, which run down to the lake, contain quite mature *Acacia tortilis* and *A. seyal*, often overgrown with *Salvadora persica* and *Capparis* spp., in groves close to freshwater inflows and springs and *A. mellifera* in the drier areas. The

Table 3. Taxonomic list for ground-layer and low-growing shrub vegetation within 100 m of the lakeshore in Lake Bogoria National Reserve, August 2001 & 2002. Nomenclature follows Agnew & Agnew (1994). Grasses not found in this study but recorded by Onkware (1996) are marked with an asterisk

- Acanthaceae
  - *Hypoestes aristata* (Vahl) Roem. & Schultes
  - *Crossandra mucronata* Lindau
  - *Barleria spinisepala* E.A. Bruce
- Aizoaceae
  - *Mollugo nudicaulis* Lam.
- Amaranthaceae
  - *Achyranthes aspera* L.
  - *Sericocomopsis hildebrandtii* Schinz
- Asclepiadaceae
  - *Calotropis procera* (Ait.) Ait.f.
- Boranginaceae
  - *Heliotropium steudneri* var. *steudneri*
- Capparaceae
  - *Maerua decumbens* (Brongn.) De Wolf
  - *Capparis tomentosa* Lam.
- Commelinaceae
  - *Commelina petersii* Hassk.
- Compositae (Asteraceae)
  - *Kleinia squarrosa* Cuf.
- Euphorbiaceae
  - *Acalypha fruticosa* Forsk.
  - *A. psilostachya* Hochst.
  - *A. racemosa* Baill.
  - Ricinus communis L.
- Labiatae
  - *Leonotis nepetifolia* (L.) Ait.f. var. africana
  - *Leucas* sp.
- Liliiflorae (Amaryllidaceae)
  - *Scilla hyacinthia* (Roth.) Alston
- Liliiflorae (Asparagaceae)
  - *Asparagus racemosus* Willd.
- Malvaceae
  - *Abutilon fruticosum* Guill. & Perr.
  - *A. mauritianum* (Jacq.) Medic.
  - *Hibiscus meyeri* Harv.
  - *H. vitifolius* L.
  - *Pavonia patens* (Andr.) Chiov.
- Orobanchaceae
  - *Orobanche minor* Smith
- Papilionaceae
  - *Indigofera volkensii* Taub.
  - *Vigna schimperi* Bak.
- Pedaliaceae
  - *Sesamum latifolium* Gillett
- Plumbaginaceae
  - *Plumbago zeylanica* L.

Table 3. Continued

- Portulacaceae
  - *Portulaca foliosa* Ker-Gawl.
  - *P. kermesina* N.E.Br.
  - *P. quadrifida* L.
- Scrophulariaceae
  - *Cycnium tubulosum* (L.f) Engl.
  - *Striga gesnerioides* (willd.) Vatke
- Solanaceae
  - *Lycium europaeum* L.
  - *Solanum incanum* L.
  - *S. nigrum* L.
  - *S. renschii* Vatke
- Vitaceae
  - *Cissus quadrangularis* L.
  - *C. rotundifolia* (Forsk.) Vahl
- Verbinaceae
  - Lantana camara (exotic)
- Zygophyllaceae
  - *Tribulus terrestris* L.
  - *Tribulus cistoides* L.
- Poaceae (grasses)
  - *Andropogon* sp.*
  - *Aristida dimuta*
  - *A. keniensis**
  - *A. somalensis*
  - *A. stenostachya*
  - *Bouteloua* sp.
  - *Brachiaria* sp.*
  - *Chenchrus ciliaris**
  - *Chloris gayana*
  - *C. virgata*
  - *Cynodon dactylon*
  - *Cynodon nlemfuensis**
  - *Dactyloctenium australe*
  - *D. bogdanii**
  - *Elusine* sp.
  - *Enteropogon rupestris**
  - *Eragrostis* sp.
  - *Heteropogon contortus*
  - *Paspalum* sp.
  - *Pennisetum* sp.
  - *Rhynchelytrum repens**
  - *Sporobolus consimilis*
  - *S. keutrophyllus**
  - *S. spicatus*
  - *Tragus berteronianus**
  - *Typha domingensis*
  - *Urochloa raceme*
- Cyperaceae (sedges)
  - *Cyperus cyperoides* L. Kuntze
  - *C. laevigatus* L.
  - *C. rubicundus* Vahl
  - *Schoenoplectus corymbosus* (Roth. ex Roem. & Schult.) J. Raynal var. *brachyceras* (A. Rich.) K. Lye Rottb

acacias had a distinct coleopteran fauna in which the Buprestidae, Chrysomelidae and Curculionidae predominated. Some species infested the trees in population outbreaks, such as occurred in February 2000, when the eumolpine chrysomelid *Malegia affinis* Jacoby was the most abundant insect on the lake shore acacias.

The bird fauna consisted of 223 species recorded on the reserve between 2000 and 2003 and although most are not associated directly with the aquatic ecosystem, just over 50 species are directly dependent upon the lake and its associated mudflats, springs, streams, seepages and wetlands (Table 4). Up to another 50 terrestrial insectivorous species feed, in part, on the adult *Paratendipes* sp. when they settle on lakeshore scrub. Two aquatic species, are wholly also dependent upon the *Paratendipes* sp. and are more abundant at this lake than any other in Kenya; Cape teal *Anas capensis* and black-necked grebe *Podiceps nigricollis*. Both feed on the emerging pupae (grebe dive for them as they rise) and adults (teal dabble on the surface film collecting adults and shed pupal exuviae). The numbers of these species fluctuated considerably, but in August 2001, 2084 *P. nigricollis* and 663 *A. capensis* were counted. The families Apodidae (swifts) and Hirundinidae (swallows and martins), which have five and seven species respectively, fed extensively on emerging adults over the water. Conservative estimates, made in February 2000 and in February 2003, suggested the numbers of these two families over the lake at least matched those of *P. minor* on it. The lake is an important feeding station for four Palaearctic migrants, in both directions, being at the southern edge of Kenya's extensive northern arid district, particularly *Hirundo rustica, Riparia riparia* and *Apus apus*.

Discussion

The lake's chemical data suggest a more stable environment than has been recorded for the other saline, more shallow, lakes in Kenya, with no evidence for the major changes recorded in Elmenteita (Melack, 1988) and Nakuru (Vareschi, 1982) caused by drying-out. A 50% change in conductivity accompanied by little pH or alkalinity variations suggests that the measured fluctuations of an order of magnitude in *A. fusiformis* is more due to biological regulation rather than environmental effects, and it is close to its theoretical maximum, as has been recorded for deep crater lakes

Table 4. Taxonomic list of birds recorded at Lake Bogoria National Reserve, 2000-3, Family, scientific and English names

- Struthionidae: Ostriches
    - Common Ostrich *Struthio camelus*
- Podicipedidae: Grebes
    - Little Grebe *Tachybaptus ruficollis*
    - Black-necked Grebe *Podiceps nigricollis*
- Pelicanidae: Pelicans
    - Great White Pelican *Pelecanus onocrotalus*
- Phalacrocoracidae: Cormorants
    - Long-tailed Cormorant *Phalacrocorax africanus*
- Ardeidae: Herons and Egrets
    - Cattle Egret *Bubulcus ibis*
    - Yellow-billed Egret *Mesophoyx intermedia*
    - Little Egret *Egretta garzetta*
    - Great Egret *Casmerodius albus*
    - Striated (Green-backed) Heron *Butorides striatus*
    - Grey Heron *Ardea cinerea*
    - Black-headed Heron *Ardea melanocephala*
- Scopidae: Hamerkop
    - Hamerkop *Scopus umbretta*
- Ciconiidae: Storks
    - Yellow-billed Stork *Mycteria ibis*
    - Wooly-necked Stork *Ciconia episcopus*
    - Marabou Stork *Leptoptilos crumeniferus*
    - White stork *Ciconia ciconia*
- Threskiornithidae: Ibises and Spoonbills
    - Sacred Ibis *Threskiornis aethiopicus*
    - Hadada Ibis *Bostrychia hagedash*
    - Glossy Ibis *Plegadis falcinellus*
    - African Spoonbill *Platalea alba*
- Phoenicopteridae: Flamingos
    - Greater Flamingo *Phoenicopterus ruber*
    - Lesser Flamingo *Phoeniconaias minor*
- Anatidae: Ducks & Geese
    - Egyptian Goose *Alopochen aegyptiacus*
    - Spur-winged Goose *Plectropterus gambensis*
    - Knob-billed Duck *Sarkidiornis melanotos*
    - White-faced Whistling Duck *Dendrocygna viduata*
    - Cape Teal *Anas capensis*
- Accipitridae: Kites, Vultures, Eagles and Hawks
    - Yellow-billed Kite *Milvus parasiticus*
    - White backed vulture *Gyps africanus*
    - African Fish Eagle *Haliaeetus vocifer*
    - African Marsh Harrier *Circus ranivorus*
    - Montagu's Harrier *Circus pygargus*
    - Eurasian Marsh Harrier *Circus aeruginosus*
    - Pallid Harrier *Circus macrourus*
    - Dark Chanting Goshawk *Melierax metabates*
    - Gabar Goshawk *Micronisus gabar*

Continued on p. 271

Table 4. Continued

- Black-chested snake eagle *Circaetus pectoralis*
- African Harrier-Hawk *Polyboroides typus*
- Augur Buzzard *Buteo augur*
- Common Buzzard *Buteo buteo*
- Tawny Eagle *Aquila rapax*
- Steppe Eagle *Aquila nipalensis orientalis*
- Verreaux's Eagle *Aquila verreauxii*
- Martial Eagle *Polemaetus bellicosus*

- Falconidae: Falcons
    - Pygmy Falcon *Polihierax semitorquatus*
    - Peregrine Falcon *Falco peregrinus*
    - Lanner falcon *Falco biarmicus*
    - Eurasian Hobby *Falco subbuteo*
- Numididae: Guineafowls
    - Helmeted Guineafowl *Numida meleagris*
- Phasianidae: Quails and Francolins
    - Jackson's Francolin *Francolinus jacksoni*
    - Crested Francolin *Francolinus sephaena*
    - Common Quail *Coturnix coturnix*
- Rallidae: Crakes and Rails
    - Black Crake *Amaurornis flavirostris*
- Gruidae: Cranes
    - Grey-crowned Crane *Balearica regulorum*
- Recurvirostridae: Avocets and Stilts
    - Black-winged Stilt *Himantopus himantopus*
    - Pied Avocet *Recurvirostra avosetta*
- Charadridae: Plovers
    - Grey plover *Pluvialis squatarola*
    - Spur-winged Lapwing *Vanellus spinosus*
    - Crowned Lapwing *Vanellus coronatus*
    - Black-headed Lapwing *Vanellus tectus*
    - Kittlitz's Plover *Charadrius pecuarius*
    - Three-banded Plover *Charadrius tricollaris*
    - Common Ringed Plover *Charadrius hiaticula*
    - Chestnut banded plover *Charadrius pallidus*
- Scolapacidae: Sandpipers and Snipes
    - Lesser Sandpiper *Charadrius mongolus*
    - Common Sandpiper *Actitis hypoleucos*
    - Ruff *Philomachus pugnax*
    - Wood Sandpiper *Tringa glareola*
    - Green Sandpiper *Tringa ochropus*
    - Common Greenshank *Tringa nebularia*
    - Marsh Sandpiper *Tringa stagnatilis*
    - Spotted Redshank *Tringa erythropus*
    - Little Stint *Calidris minuta*
    - Curlew Sandpiper *Calidris ferruginea*
    - Common Snipe *Gallinago gallinago*
    - Broad-billed sandpiper *Limicola falcinellus*
- Pteroclidae: Sandgrouse
    - Lichtenstein's Sandgrouse *Pterocles lichtensteinii*

Table 4. Continued

- Columbidae: Pigeons and Doves
    - African Green-Pigeon *Treron calva*
    - Speckled Pigeon *Columba guinea*
    - Emerald-spotted Wood-Dove *Turtur chalcospilos*
    - Namaqua Dove *Oena capensis*
    - Ring-necked Dove *Streptopelia capicola*
    - Red-eyed Dove *Streptopelia semitorquata*
    - African Mourning Dove *Streptopelia decipiens*
    - Laughing Dove *Streptopelia senegalensis*
- Musophagidae: Turacos
    - White-bellied Go-away-bird *Corythaixoides leucogaster*
- Cuculidae: Cuckoos and Coucals
    - Klaas's cuckoo *Chrysococcx klaas*
    - White-browed Coucal *Centropus superciliosus*
- Strigidae: Owls
    - African Scops-Owl *Otus senegalensis*
    - Verreaux's Eagle-Owl *Bubo lacteus*
    - Pearl-spotted Owlet *Glaucidium perlatum*
- Caprimulgidae: Nightjars
    - Slender-tailed nightjar *Caprimulgus clarus*
- Apodidae: Swifts
    - Little Swift *Apus affinis*
    - White-rumped Swift *Apus caffer*
    - Mottled Swift *Apus aequatorialis*
    - Nyanza Swift *Apus niansae*
    - Eurasian Swift *Apus apus*
- Coliidae: Mousebirds
    - Speckled Mousebird *Colius striatus*
    - Blue-naped Mousebird *Urocolius macrourus*
    - Red-faced Mousebird *Urocolius indicus*
- Alcedinidae: Kingfishers
    - Grey-headed Kingfisher *Halcyon leucocephala*
    - Woodland Kingfisher *Halcyon senegalensis*
    - Malachite Kingfisher *Alcedo cristata*
    - African Pigmy Kingfisher *Ispidina picta*
- Meropidae: Bee Eaters
    - Little Bee eater *Merops pusillius*
    - Cinnamon-chested Bee eater *Merops oreobates*
    - European Bee eater *Merops apiaster*
    - Madagascar Bee eater *Merops superciliosus*
    - White-fronted Bee eater *Merops bullockoides*
- Coraciidae: Rollers
    - Lilac-breasted Roller *Coracias caudate*
    - Rufous-crowned Roller *Coracias naevia*
- Upupidae: Hoopoes
    - African Hoopoe *Upupa africana*
- Phoenuculidae: Wood-hoopoes
    - Green Wood-hoopoe *Phoeniculus purpureus*

Continued on p. 272

Table 4. Continued

- Bucerotidae: Hornbills
  - Red-billed Hornbill *Tockus erythrorhynchus*
  - Von der Decken's Hornbill *Tockus deckeni*
  - Jackson's Hornbill *Tockus jacksoni*
  - African Grey Hornbill *Tockus nasutus*
- Captionidae: Barbets and Tinkerbirds
  - Red-fronted Tinkerbird *Pogoniulus pusillus*
  - Red-fronted Barbet *Tricholaema diademata*
  - Black-throated Barbet *Tricholaema melanocephala*
  - White-headed Barbet *Lybius leucocephalus*
  - d'Arnaud's Barbet *Trachyphonus darnaudii*
  - Red and yellow Barbet *Trachyphonus erythrocephalus*
- Indicatoridae: Honeyguides:
  - Lesser Honeyguide *Indicator minor*
- Picidae: Woodpeckers
  - Nubian Woodpecker *Campethera nubica*
  - Cardinal Woodpecker *Dendropicos fuscescns*
  - Bearded Woodpecker *Dendropicos namaquus*
  - Grey Woodpecker *Dendropicos goertae*
- Alaudidae: Larks
  - Fischer's Sparrow-Lark *Eremopterix leucopareia*
- Hirundinidae: Swallows and martins
  - Rock Martin *Hirundo fuligula*
  - Plain Martin *Riparia paludicola*
  - Sand Martin *Riparia riparia*
  - Red-rumped Swallow *Hirundo daurica*
  - Lesser Striped Swallow *Hirundo abyssinica*
  - Barn Swallow *Hirundo rustica*
  - Wire-tailed Swallow *Hirundo smithii*
  - Mosque Swallow *Hiundo senegalensis*
- Motacillidae: Pipits and Wagtails
  - African Pied Wagtail *Motacilla aguimp*
  - Yellow-headed Wagtail *Motacilla lutea*
  - Yellow wagtail *Motacilla flava*
  - White wagtail *Motacilla alba*
- Pycnonotidae: Bulbuls
  - Common Bulbul *Pycnonotus barbatus*
- Turdidae: Thrushes
  - African Thrush *Turdus pelios*
  - Olive Thrush *Turdus olivaceous*
  - Spotted Morning Thrush *Cichladusa guttata*
  - White-browed Scrub Robin *Cercotrichas guttata*
  - Isabelline Wheatear *Oenanthe isabellina*
  - Pied Wheatear *Oenanthe pleschanka*
  - Northern Wheatear *Oenanthe oenanthe*
- Sylvidae:Warblers
  - Olivaceous Warbler *Hippolais pallida*
  - Barred Warbler *Sylvia nisoria*

Table 4. Continued

  - Willow Warbler *Phylloscopus trochilus*
  - Yellow-breasted Apalis *Apalis flavidus*
  - Red-face Cromec *Sylvieta whytii*
  - Grey-backed Camaroptera *Camaroptera brachyura*
- Muscicapidae: Flycatchers
  - Southern Black Flycatcher *Melaenornis pammelaina*
  - African Grey Flycatcher *Bradornis microrhynchus*
  - Spotted Flycatcher *Muscicapa striata*
  - Silverbird *Empidornis semipartitus*
- Platysteiridae: Batises
  - Pygmy Batis *Batis perkeo*
- Monarchidae: monarch flycatchers
  - African Paradise-flycatcher *Terpsiphone viridis*
- Timaliidae: Chatterers and Babblers
  - Rufus Chatterer *Turdoides rubiginosus*
  - Northern Pied Babbler *Turdoides hypoleucus*
- Paridae: Tits
  - White-bellied Tit *Parus albiventris*
  - Northern Grey Tit *Parus thruppi*
  - Red-throated Tit *Parus fringillinus*
- Nectariniidae: Sunbirds
  - Beautiful Sunbird *Cinnyris pulchella*
  - Eastern Violet-backed Sunbird *Anthreptes orientalis*
- Laniidae: Shrikes
  - Common Fiscal *Lanius collaris*
  - Long-tailed Fiscal *Lanius cabanisi*
  - Grey-backed Fiscal *Lanius excubitoroides*
- Malaconotidae: Bush Shrikes
  - Tropical Boubou *Laniarius aethiopicus*
  - Slate-coloured Boubou *Laniarius funebris*
  - Brubru *Nilaus afer*
  - Black-backed Puffback *Dryoscopus cubla*
  - Northern Puffback *Dryoscopus gambensis*
- Prionopidae: Helmet-shrikes
  - Northern White-crowned Shrike *Eurocephalus rueppelli*
- Dicruridae: Drongos
  - Fork-tailed Drongo *Dicrurus adsimilis*
- Corvidae: Crows
  - Pied Crow *Corvus albus*
- Oriolidae: Orioles
  - African Black-headed Oriole *Oriolus larvatus*
  - African Golden Oriole *Oriolus auratus*
- Sturnidae: Starlings and Oxpeckers
  - Rüppell's Long-tailed Starling *Lamprotornis purpuropterus*
  - Supurb Starling *Lamprotornis superbus*
  - Greater Blue-eared Starling *Lamprotornis chalybaeus*
  - Abbot's Starling *Cinnyricinclus femoralis*
  - Ashy Starling *Cosmopsarus unicolor*
  - Magpie Starling *Speculipastor bicolour*

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Table 4. Continued

- Wattled Starling *Creatophora cinerea*
- Red-billed Oxpecker *Buphagus erythrorhynchus*

Passeridae: Sparrows
- House Sparrow *Passer domesticus*
- Chestnut Sparrow *Passer eminibey*
- Grey-headed Sparrow *Passer griseus*
- Rufous Sparrow *Passer rufocinctus*
- Yellow-spotted Petronia *Petronia pyrgitta*

Ploceidae: Weavers
- White-headed Buffalo-Weaver *Dinemellia dinemelli*
- Speckle-fronted Weaver *Sporopipes frontalis*
- Red-headed Weaver *Anaplectes rubriceps*
- White-billed Buffalo-Weaver *Bubalornis albirostris*
- Red-billed Buffalo Weaver *Bubalornis niger*
- White-browed Sparrow-Weaver *Plocepasser mahali*
- Northern Masked Weaver *Ploceus taeniopterus*
- Vitelline Masked Weaver *Ploceus velatus*
- Reichenow's Weaver *Ploceus baglafecht*
- Speke's Weaver *Ploceus spekei*
- Golden-backed Weaver *Ploceus jacksoni*
- Black-headed Weaver *Ploceus cucullatus*
- Little Weaver *Ploceus luteolus*

Emberizidae: Waxbills and Whydahs
- Green-winged Pytilia *Pytilia melba*
- Red-cheeked Cordon-bleu *Uraeginthus bengalus*
- Blue-capped Cordon-bleu *Uraeginthus cyanocephalus*
- Purple Grenadier *Uraeginthus ianthinogaster*
- Red-billed Firefinch *Lagonosticta senegala*
- Black-cheeked Waxbill *Estrilda charmosyna*
- Pin-tailed Whydah *Vidua macroura*
- Straw-tailed Whydah *Vidua fischeri*
- Steel-blue Whydah *Vidua hypocherina*
- Village Indigobird *Vidua chalybeata*

Fringillidae: canaries and seadeaters
- African Citril *Serinus citrinelloides*
- Streaky Seedeater *Serinus striolatus*
- White-bellied Canary *Serinus dorsostriatus*

in Ethiopia such as Lake Aranguadi (Talling et al., 1973).

The monoculture of *A. fusiformis* in the phytoplankton, the absence of any macro-zooplankton, and a single species in the benthos, reflect the extreme saline environment. This implies instability, using the 'simple-unstable, diverse-stable' concept of ecology espoused in the 1970s. The initial data presented here however, indicates that a hydro-chemical stability confers an ecological stability on an extremely simple limnological food-web. The greatest instability in the lake was the outbreak of mortality of *P. minor*. Periodic large-scale die-offs have been reported for the past 40 years. Initially, these die-offs were attributed to starvation, due to sudden crashes of phytoplankton and other unknown causes (Brown, 1959). At Lake Nakuru, during the first three months of 1974, the 75% decline in the standing crop of *A. fusiformis*, was followed by a subsequent massive emigration of lesser flamingos from > one million birds in January to < 10 000 by August–September (Tuite, 2000). Amongst the approximately 10 000 birds remaining during July and August, there was unusually high mortality, 420 carcasses being collected in 19 days (Sileo et al., 1979). In April, 51 debilitated birds (26 adults and 25 immature birds) were collected for analysis. Ten adults (38%) and nine immature birds (36%) had avian tuberculosis *Mycobacterium avium* Type 1 (Sileo et al., 1979). The rest perhaps were debilitated from a combination of starvation and the effects of parasites.

There have been three notable die-offs within the past decade, in 1993, 1995, and again in 1999–2000. The 1993 die-off, which was first noticed at Lake Bogoria and then a month later at Lake Nakuru, resulted in an estimated 18 500 deaths on the two lakes. Zimbabwe veterinarians Nancy and Richard Kock studied this die-off, which occurred at a time when the population was unusually high, water levels declining, and ambient air temperatures high (Nasirwa & Bennun, 1994; Kock et al., 1999). They concluded that the primary cause of death was "septicemia, complicated in those affected, by mycobacteriosis", or avian tuberculosis (Kock et al., 1999).

M. avium Type 1 was first isolated in a *P. minor* in Kenya at Lake Nakuru in 1970 by Koeman et al. (1972). The African fish eagle *Haliaeetus vocifer* feeds largely on *P. minor* there (Cooper et al., 1975) and in 1972, an *H. vocifer* at Lake Nakuru was also found infected with the disease (Kaliner & Cooper, 1973). In 1973, Cooper et al. (1975) had found *M. avium* Type 1 in two of four debilitated *P. minor* also collected at Lake Nakuru. The species had been shown not to be very susceptible to avian TB in captive flocks held within avian collections where avian TB was otherwise prevalent (Wood, 1975), so it was not clear why they seemed so susceptible at Lake Nakuru, or how the disease was introduced there.

One hypothesis is that the bacterium might have been introduced to Lake Nakuru as an effect of ecological changes following the introduction of *Tilapia grahami* in the early 1960s and the immigration of fish eating birds such as the pelican (Cooper et al., 1975). Its spread to other lakes could then have been accomplished by the flamingos themselves through their constant vagrancy. In humans, susceptibility to tuberculosis increases amongst underweight and undernourished individuals living in overcrowded conditions (Evans & Feldman, 1982; Benenson, 1990). Ratcliffe (1946) studied data from 3000 avian postmortems performed over a 20-year period at the Philadelphia Zoological Garden and concluded that resistance of birds to tuberculosis was influenced by nutritional factors. Perhaps, the dense flocks of hungry *P. minor* at lakes Nakuru and Bogoria following a phytoplankton decline, provide the conditions for *M. avium* to become epizootic; at Bogoria the number of birds reached nearly a million just prior to each of the three mortalities (Owino et al., 2001). On the other hand, in some humans and laboratory animals, genetic factors have been shown to influence resistance to the disease (Evans & Feldman, 1982). Wood (1975) suggested that similar factors might operate in flamingos, based on his observations of captive birds.

A second hypothesis to explain the mass lesser flamingo die-offs is that they are the result of toxicosis. A joint study by the World Wide Fund for Nature and Egerton University found substantial amounts of hepatoxic algae and toxic metals, particularly chromium, in Lake Nakuru that appears to have originated from industries surrounding the lake. Web-site claims were made for high levels in bird tissues although these have not yet been supported by scientific publication. A linked experimental study predicted increased metal exposure rates through increased absorption by birds as the population of *A. fusiformis* declined (Nelson et al., 1998), which provided a possible explanation for the die-offs observed at Lake Nakuru. Toxicosis is not yet clearly a cause of flamingo deaths at Lake Nakuru without hard evidence, but cannot be ruled out. In Lake Bogoria however, where the 1993 die-off was first noticed and the 1999–2000 was almost entirely confined, there is no polluting industry in its catchment.

The pathological role of algal toxins in flamingos has also been suggested as a contributing factor at Bogoria (Krienitz et al., 2003), where toxin-secreting species were identified in hot-spring streams and in two flamingo carcasses in 2001, the period of the lower mortality-peak (see above). In greater flamingos, cyanobacterial toxins from planktonic species known to be toxin-generating – *Microcystis aeruginosa* and *Anabaena flos-aquae* – have been identified as the causative agents for a mortality of 60% of *Phoenicopterus ruber* chicks in Doñana National Park, southern Spain, in 2001. At Bogoria, the water of hot spring streams is potable if its salinity is substantially lower than lake water (conductivity by up to one-third lower, pers. obs. DMH). Lines of birds may be seen drinking from such streams (e.g. Fig. 1E in Krienitz et al., 2003). However, the water represents a small proportion of the available drinking water at Bogoria and hot spring sources are primarily located along the middle of the western shore. In the north, the Sandai river brings a small permanent flow of the diverted Loboi river and in the south, two freshwater springs supply permanent streams; all three are used extensively for drinking (DMH, RBC, MMH, pers. obs.).

In all probability there are multiple causes of the mortality. Avian tuberculosis may be endemic in the population but not lethal to individuals unless their immunity has been compromised by another factor. It is perhaps not an accident that all three mortalities in the 1990s occurred towards the end of long droughts (LaVigne & Ashley, 2001) as well as at a time when the number of birds at Bogoria peaked at close to 1×10^6 (Owino et al., 2001), both factors which would have increased stress on individuals. The population in 2000 might also have been undernourished, since the quantity of *A. fusiformis* was low and the filtering demands of *P. minor* are high (Vareschi, 1978).

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Sulfate inhibition of molybdenum-dependent nitrogen fixation by planktonic cyanobacteria under seawater conditions: a non-reversible effect

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Abstract

The trace element molybdenum is a central component of several enzymes essential to bacterial nitrogen metabolism, including nitrogen fixation. Despite reasonably high dissolved concentrations (for a trace metal) of molybdenum in seawater, evidence suggests that its biological reactivity and availability are lower in seawater than in freshwater. We have previously argued that this difference is related to an inhibition in the uptake of molybdate (the thermodynamically stable form of molybdenum in oxic natural waters) by sulfate, a stereochemically similar ion. Low molybdenum availability may slow the growth rate of nitrogen-fixing cyanobacteria, and in combination with an ecological control such as grazing by zooplankton, keep fixation rates very low in even strongly nitrogen-limited coastal marine ecosystems. Here we present results from a seawater mesocosm experiment where the molybdenum concentration was increased 10-fold under highly nitrogen-limited conditions. The observed effects on nitrogen-fixing cyanobacterial abundance and nitrogen-fixation inputs were much smaller than expected. A follow-up experiment with sulfate and molybdenum additions to freshwater microcosms showed that sulfate (at seawater concentrations) greatly reduced nitrogen fixation by cyanobacteria and that additions of molybdenum to the levels present in the seawater mesocosm experiment only slightly reversed this effect. In light of these results, we re-evaluated our previous work on the uptake of radio-labeled molybdenum by lake plankton and by cultures of heterocystic cyanobacteria. Our new interpretation indicates that sulfate at saline estuarine levels ($>$8–10 mM) up to seawater (28 mM) concentrations does inhibit molybdenum assimilation. However, the maximum molybdenum uptake rate ($V_{\max}$) was a function of the sulfate concentration, with lower $V_{\max}$ values at higher sulfate levels. This indicates that this inhibition is not fully reversed at some saturating level of molybdenum, as assumed in a simple competitive inhibition model. A multi-enzyme, mixed kinetics model with two or more uptake enzyme systems activated in response to the environmental sulfate and molybdate conditions may better explain the repressive effect of sulfate on Mo-mediated processes such as nitrogen fixation.

Introduction

Molybdenum (Mo), although only required in trace amounts, is a central component of several enzymes essential to bacterial nitrogen (N) metabolism, including the enzymes for assimilatory and dissimilatory reduction of nitrate (nitrate reductase) and for the fixation of atmospheric N_2 to ammonium (nitrogenase; Brill & Shah, 1980; Spiro, 1985; Cole et al., 1986). This process of biological N_2-fixation is an essential pathway by which new N enters the biosphere and supports the production of organic carbon.

Since the 1800s when N_2 fixation was first hypothesized to occur in leguminous plants (Schrauzer, 1976), there has been great interest in elucidating the controls on this extraordinary process. It is particularly intriguing that annual rates of primary production in some ecosystems are often limited by N availability despite the widespread occurrence of N_2-fixing bacteria (Vitousek & Howarth, 1991; Vitousek et al., 2002). The apparent dichotomy in the significance of planktonic N_2 fixation in response to N deficits in many estuarine/coastal marine systems as compared to freshwater lakes is an interesting example that has stimulated much research (see reviews in Howarth et al., 1988a; Paerl, 1996; NRC, 2000). Nitrogen fixation by planktonic cyanobacteria is often an important process in freshwater lakes, where it helps alleviate deficits in N compared to phosphorus (P) and thus maintains P limitation of net primary production (Schindler, 1977; Howarth, 1988). In sharp contrast, planktonic N_2 fixation rarely occurs in most estuaries even when these systems are strongly N limited (Howarth et al., 1988a; NRC, 2000; Marino et al., 2002).

The physiological role of Mo in biological N_2fixation has long been recognized (Bortels, 1930; Fogg & Wolfe, 1954). The possibility that low Mo availability may be a factor limiting production and N_2 fixation in some oligotrophic lakes with low concentrations of Mo (<0.6 nM) was suggested by Hutchinson (1957) and Goldman (1960, 1964). The range of Mo concentrations reported in freshwaters varies widely, and while often low can be as high or higher than that of seawater (107 nM; Bradford et al., 1968; Manheim & Landergren, 1978; Collier, 1985; Howarth et al., 1988b; Marino et al., 1990). In his classic study of the natural cycle of Mo in a shallow eutrophic lake (Lake Donk), Dumont (1972) demonstrated a dynamic seasonal pattern. A winter depletion of Mo due to binding in sediments was followed by a large release to the water column in early summer, and then a gradual decline in concentration over the summer growing season due to uptake by plankton, including the abundant N_2-fixing cyanobacteria. The peak summer-season dissolved Mo concentrations were roughly equivalent to those found in ocean waters (Dumont, 1972). In general, both seasonally-averaged and individual measurements of the amount of Mo found in the phytoplankton of this eutrophic lake were comparable to the amount of Mo present in the dissolved phase (particulate to dissolved Mo ratio of 0.25–0.7; Dumont, 1972). A seasonal decline in euphotic-zone Mo concentrations concurrent with a N_2-fixing cyanobacteria bloom, and a roughly equal partitioning of Mo between the seston and dissolved forms have subsequently been reported in other freshwater ecosystems (Cowgill, 1976; Howarth et al., 1988b).

The behavior of Mo in oceanic systems appears to be quite different. Mo is a conservative element in seawater, with little if any depletion due to biotic uptake (Manheim & Landergren, 1978; Howarth et al., 1988b; Tuit, 2003). The amount of Mo dissolved in seawater is some 5000–25 000-fold greater than Mo concentrations found in seston (Berrang & Grill, 1974; Manheim & Landergren, 1978; Howarth et al., 1988b; Tuit, 2003, with assumption of 1 mg l^{-1} seston as in Howarth et al., 1988b). For metals with a high bioavailability, the concentration in the particulate phase is typically similar to or greater than that in the dissolved phase (Morel & Hudson, 1985). Somewhat surprisingly, while the concentration of dissolved Mo in seawater is orders of magnitude higher than that of biologically reactive metals such as iron, manganese, zinc, copper, nickel, or cadmium, the concentration of Mo in the seston of oceanic systems is lower than that of these other metals (Manheim & Landergren, 1978; Collier & Edmond, 1984; Howarth et al., 1988b; Sunda, 1989). This strong partitioning of Mo into the dissolved pool in oceanic systems suggests a very low biotic reactivity in seawater.

We (Howarth & Cole, 1985) hypothesized that the higher concentrations of sulfate in seawater may increase the energetic cost of assimilating Mo in coastal marine systems, and in particular slow rates of N_2fixation and so the growth of N_2-fixing cyanobacteria in N-deficient estuaries compared to freshwaters. Molybdenum is unusual for a trace metal in that it is present in oxic waters primarily as an anion (molybdate). We suggested that the observed low reactivity and biotic availability of Mo in seawater compared to freshwaters is the result of the sulfate anion interfering with the assimilation of molybdate (Howarth & Cole, 1985). Sulfate and molybdate are remarkably similar in their stereochemistry and effective size (Cotton & Wilkinson, 1972), making it potentially difficult for an uptake enzyme to discriminate between the two unless there is very high specificity for molybdate. Sulfate is the second most abundant anion in seawater, and the ratio of sulfate to Mo concentration is 1–2 orders of magnitude greater than in most freshwaters (Howarth et al., 1988b; Marino et al., 1990). Empirical evidence from a field study of 13 saline lakes with independently varying sulfate and Mo concentrations demonstrated that the ratio of sulfate to Mo proved to be the best predictor of the abundance of N-fixing cyanobacteria over the summer season (Marino et al., 1990). Molybdenum uptake experiments using radioactive ^{99}Mo have demonstrated that sulfate does indeed interfere with the assimilation of molybdate by phytoplankton and bacteria growing on nitrate and by N-fixing cyanobacteria in both cultures and natural populations (Cole et al., 1986, 1993). Inhibition of Mo assimilation by sulfate has also been shown in a variety of other organisms, such as tomato plants (Stout & Meagher, 1948), animal intestines (Huising & Matrone, 1975; Cardin & Mason, 1976), and the bacteria *Clostridium pasteurianum* (Elliot & Mortenson, 1975) and *E. coli* (Corcuera et al., 1993; Grunden & Shanmugam, 1997). All of these Mo-assimilation studies concluded that Mo uptake was the result of an active, energy-requiring process rather than an abiotic process such as surface sorption.

In our Mo uptake studies, we found that ^{99}Mo-labelled molybdate assimilation followed saturation-type kinetics, and we fit the data to a model that assumed sulfate acts as a competitive inhibitor of molybdate uptake by a single enzyme (Howarth & Cole, 1985; Cole et al., 1986, 1993; Howarth et al., 1988b). An important assumption of the competitive inhibition model is that the effect of the inhibitor is completely reversed when the enzyme becomes saturated at high enough substrate concentrations (Fromm, 1983). Therefore, increased inhibition of Mo uptake due to increasing sulfate levels in the environment should slow cyanobacterial growth relative to that when no sulfate is present at the same Mo concentration, but the same maximum Mo uptake rate should eventually be reached under both high and low sulfate conditions. The effect of the inhibitor (sulfate) on substrate (molybdate) uptake in the competitive inhibition model is to modify the effective half-saturation constant for molybdate uptake (K_m) as a function of Mo concentration, but not to influence the maximum potential rate of molybdate uptake, or V_max, as described by the modified Michaelis-Menten equation:

$$V = V_\mathrm{max} * \mathrm{S}/[(1 + \mathrm{I}/K_\mathrm{i}) * K_\mathrm{m} + \mathrm{S}], \qquad (1)$$

where V is the uptake velocity for molybdate (nmoles l^{-1} hr^{-1}), V_max is the uptake velocity at saturating concentrations of substrate, S is the concentration of the substrate (molybdate, nM), I is the concentration of inhibitor (sulfate, mM), K_i is an inhibition constant (mM), and K_m is the half-saturation constant for molybdate assimilation (nM; Cole et al., 1993).

More recently, we used a form of Equation (1) and the data of Cole et al. (1993) in a simulation model examining the interactive effects of Mo availability and ecological controls such as grazing pressure on the development of cyanobacterial blooms and associated N_2 fixation in estuaries and in lakes (Howarth et al., 1999). We concluded from that work that a slow growth rate of heterocystic cyanobacteria under high sulfate conditions interacts with grazing to limit strongly the development of N-fixing cyanobacterial blooms, as well as the importance of N_2 fixation as a N input, in saline estuarine systems (Howarth et al., 1999; Marino et al., 2002). We next conducted a series of seawater mesocosm experiments designed to test the basic predictions of the Howarth et al. (1999) model. We have previously described the results of a subset of these experiments with regard to the effects of grazing on cyanobacterial growth and N_2 fixation (Marino et al., 2002). Here we present results from an aspect of those experiments not previously reported, addressing the effect of an experimental addition of Mo to 10 times ambient seawater concentration. We also present results from a follow-up, short-term microcosm-scale experiment designed to further explore the specific effect of sulfate on N_2 fixation using a natural population of N_2-fixing cyanobacteria from a nutrient-enriched freshwater pond. These results taken together led us to a re-analysis and partial re-interpretation of our previous Mo uptake work.

Materials and methods

Mesocosm Mo addition experiment

We included a Mo manipulation in a mesocosm experiment conducted in 1998 at the University of Rhode Island, as part of a 2 × 2 matrix design with grazing. There were a total of 16 mesocosms, with 4 replicates of each treatment. The general experimental setup is summarized briefly below; further details of the site, experimental design, and methods used are given in Marino et al. (2002) and Marino (2001).

Mesocosms were 3 m^3 Kalwal fiberglass tanks, filled with Narragansett Bay water (salinity 27–32 ppt), open to the atmosphere and run in batch for 59 days (end date September 1, 1998). Mixing was provided at environmentally reasonable turbulence levels using bubblers (Marino et al., 2002). Phosphorus was added to all tanks twice per week at a loading rate of 160 μmol m^{-3} d^{-1}; no N was added other than what occurred in direct precipitation and in small amounts from a weekly cyanobacterial seeding source. The resulting dissolved N:P molar ratio in all tanks was well below the Redfield ratio of 16:1 throughout the experiment (Marino et al., 2002). Zooplankton grazing levels (low, denoted 'LG', and high, 'HG' treatments) were established with the presence or absence of zooplanktivorous fish (*Menidia beryllina*). Within each grazing treatment block, there were two levels of Mo, a no-addition representing ambient Narragansett Bay water (mean dissolved [Mo] of 98 nM, denoted 'LMo'), and an approximately 10X ambient addition (denoted 'HMo', 1.23 μM dissolved [Mo]). Molybdenum additions were made once, at the start of the experiment, using an acidified solution of Na_2MoO_4; Mo analyses by graphite furnace AA spectroscopy confirmed that the added Mo remained in the dissolved phase during the experiment and that there was no significant difference in Mo concentration within a block with grazing level (Marino, 2001).

Total (TN_w = dissolved + particulate), total dissolved (TDN = DON + DIN), and dissolved inorganic N (DIN = NH_4+ NO_3+ NO_2) were measured weekly on integrated water-column samples, and on an event basis in bulk precipitation, using standard techniques (Koroleff, 1983; Marino, 2001). Water column particulate (seston) N was calculated as TN_w – TDN. Particulate N in bottom floc, which was quantified at the end of the experiment, and in surface floc which was regularly removed, were measured on pooled samples taken from each mesocosm. Samples were rinsed with deionized water, frozen and freeze-dried before analysis on a Carlo-Erba CN analyzer. Data from the various N pools were used to calculate the total N increase over the entire experiment in each mesocosm ('TN increase'). Phytoplankton were sampled weekly throughout the experiment and more intensively for shorter periods of time (Chan, 2001). Samples were preserved with Lugols solution and quantified for cyanobacterial cells and heterocysts on a Wild M-40 inverted microscope at 100× and 400×, after gravitational settling in a 5-ml counting cell (Chan, 2001). Cell and heterocyst densities for each sampling were log-transformed before statistical analysis to equalize variance both within and across treatments, and to allow proper weighting of the density data over the variable time intervals of collection. Time-weighted means of the transformed data were analyzed by simple factorial ANOVA using the commercially available statistics package StatView (SAS Institute, Inc.). There was an unexplained increase in zooplankton abundance in some of the LGr mesocosms during the last two weeks of the experiment (i.e. after August 17); however, detailed data characterizing the phytoplankton biomass of the mesocosms, as well as the nutrient biogeochemistry over time, did not suggest any deviations from the treatment-specific patterns apparent prior to August 17 (Marino, 2001).

Microcosm sulfate X Mo experiment

We designed a shorter-term experiment to examine the effect of seawater-level sulfate additions (28 mM) alone and in combination with the two levels of molybdate present in the mesocosm experiment described above on rates of cyanobacterial growth and N_2 fixation by a natural assemblage of freshwater cyanobacteria. We ran the experiment in August 1999 using water from a P-fertilized freshwater pond (Pond 225, Cornell Experimental Ponds Facility) with abundant N_2-fixing *Anabaena* spp. (Chan, 2001). Pond water was first screened (145-μm mesh) to exclude macrozooplankton. Replicate 4-l polycarbonate vessels (pre-cleaned with multiple acid and DIW soakings) were set up for each of 5 treatments, described below (n = 3, for a total of 15 vessels). One liter of the screened water was mixed with two liters of 0.45-μ filtered pond water, thus diluting the phytoplankton in a proportion of 1:3 total volume. All treatments received P at the same volume-specific loading rate as the mesocosm experiment (see above); DIP concentrations were maintained at ~1 μM during the experiment. No N was added. Bicarbonate was added to all vessels (2.4 mM) to buffer the pH and guard against short-term CO_2limitation. Two control treatments were run: pond water with no further additions ('Control'), and pond water with chloride added to control for physiological effects of ionic strength or osmotic pressure ('Salt Control'). In the latter, total moles of cations and total equivalents of anion charge were kept constant with the sulfate addition treatments (below); Na^+, Mg^{2+}, and Ca^{2+} were added to the same final concentrations as in the sulfate treatments, but entirely as Cl^- salts (Ca, Mg, and Na equal to 4.5, 24, and 9 mM, respectively). Sulfate and Mo concentrations in both Control treatments remained at the ambient freshwater pond level (0.02 mM & 5.2 nM, respectively).

Sulfate was added to the remaining three treatments (denoted 'Sulfate', 'Sulfate+Mo', 'Sulfate+10X Mo') to raise the final concentration in the vessels to that of 35 ppt salinity seawater (28 mM); ACS reagent-grade Na^+ and Mg^{2+} salts were used. Calcium was also added (as $CaCl_2$) so that Ca and Mg were present at the same ratio as in seawater, and at final concentrations of 4.5 and 24 mM, respectively (45% of the concentrations in 35 ppt seawater). These additions were made to avoid the possibility of a negative sulfate response due to an induced Ca or Mg deficiency, as sulfate is the major ion which complexes with dissolved Ca^{2+} and Mg^{2+} in seawater (Stumm & Morgan, 1981). In a previous experiment using seawater from Vineyard Sound, MA, where we manipulated the SO_4:Mo ratio by elevating the ambient sulfate concentration 5-fold (to 125 mM), we observed a strong negative effect of sulfate on diatom growth using NO_3 (Marino and Howarth, unpublished data). However, when Mg and Ca were added to the 125 mM sulfate treatments in concentrations estimated using a chemical equilibrium model (MINEQL) to result in free ion activities close to those in seawater, there was no difference in phytoplankton growth, and the sulfate effect was reversed. We concluded that the sulfate effect observed in that Vineyard Sound experiment was due to complexation of Mg and/or Ca rather than any inhibitory effect on Mo uptake.

The 'Sulfate' treatment, which had an ambient (pond) dissolved Mo concentration of 5.2 nM, received no further additions. The two Mo addition treatments were designed to correspond to the concentrations of Mo and sulfate present in the seawater mesocosm experiment (see above). The 'Sulfate+Mo' treatment (corresponding to the mescosm LMo) had Mo added to a final concentration of 100 nM, or approximately that of 32 ppt seawater (Collier, 1985), and the 'Sulfate+10X Mo' treatment had Mo added to a final concentration of 1000 nM, or roughly ten times that of full-salinity seawater (corresponding to the mesocosm HMo treatment).

Treatments were incubated for 8 days in a greenhouse at 23–27 °C under natural light enhanced with full-spectrum PAR grow lights (Phillips Agro Son 430 watt); light intensity was maintained at 100–400 μE m^{-2} s^{-1} for 16-h d^{-1}. Vessels were moved randomly in the light field twice daily during hours of peak intensity and were opened and stirred manually; this was effective in limiting phytoplankton from settling and preventing wall growth. Control vessels were scanned for cyanobacterial heterocysts and cells daily during the experiment. Phytoplankton were sampled daily and handled as in the mesocosm experiment; cyanobacterial cells and heterocysts were counted in a subset of samples, as described above. Chlorophyll *a* was extracted in methanol from GF/F filtered samples at the end of the experiment and measured by fluorometry (Holm-Hansen & Riemann, 1978). Nitrogen fixation was assayed by acetylene reduction (Flett et al., 1976; Howarth et al., 1993, modified as in Marino, 2001) under experimentally similar light and temperature conditions, on day eight, when cell and heterocyst abundances in the Control treatment had undergone 3–4 doublings. Triplicate samples from each vessel were assayed for ethylene production by gas chromatography after 4-h incubations; we also assayed for dark fixation (none detected).

Results and discussion

Mo addition to estuarine mesocosms

The 1998 mesocosm experiment explored the influence of Mo additions and zooplankton grazing on planktonic, N_2-fixing cyanobacteria under saline estuarine conditions. Here we use two variables, the abundance of heterocysts over the course of the experiment, and the increase in total nitrogen in the mesocosms, to compare treatment responses. Heterocysts are the site of N_2 fixation for the cyanobacteria that grew in the mesocosms (primarily *Anabaena* sp.), and heterocyst abundances have been shown to correlate with N_2 fixation in both some natural and experimental systems, including earlier freshwater experiments in these mesocosms (Howarth et al., 1993 and references therein; Findlay et al., 1994; Marino, 2001). We have previously reported that low grazing by zooplankton had a pronounced effect on the abundance of cyanobacteria heterocysts in the mesocosms in both this (1998) experiment and in an earlier (1996) experiment (Marino et al., 2002). The relative treatment responses to Mo and grazing manipulations in this experiment can be seen in Figure 1, which shows the mean heterocyst abundance over time for each of the four treatments (note log scale). We had predicted that our Mo addition ('HMo') would largely overcome the inhibiting influence of the high sulfate levels in seawater, and that we would see a greater abundance of heterocysts in the HMo treatments, and especially in the tanks where the dominant influence of grazing on cyanobacterial abundance was eliminated. The heterocyst data hint at such a trend during the final 4 weeks of the experiment, in the mesocosms with low grazing by zooplankton (Fig. 1, 'HMo & LGr'); however, heterocyst abundances appeared to be greater in the low-grazing, no Mo-addition treatments ('LMo & LGr') during the first 2 weeks of the experiment (Fig. 1). A 2-factor ANOVA showed no significant effect of Mo on heterocyst abundance and no significant interaction of grazing and Mo addition (Table 1).

TN increase was used as an integrated response variable to assess the relative influence of Mo and grazing on cyanobacterial N_2 fixation during the entire experiment, since the N budgets for the tanks allow for the calculation of the N increase in each treatment attributable to N_2 fixation. The total nitrogen in the water column (TN_w) was the pool that showed the largest change in each treatment, increasing over time in all treatments (Fig. 2-a). Particulate (seston) N in the water column over time showed patterns very similar to the water-column TN_w (Fig. 2-b). Consistent with the heterocyst results, grazing had a highly significant influence on the total N increases in the mesocosms, and Mo additions alone were not significant. The interaction of grazing and Mo addition was significant at the 10% level (Table 1), but this interaction is not straightforward to interpret, as the Mo effect was not consistent across the grazing treatment blocks (i.e. the mean for the HMo treatment was higher than for the LMo treatment within the LGr block, but slightly lower than the LMo treatment within the HGr block; Marino, 2001). Since the input of N from atmospheric deposition was comparable across treatments, the differences in total N inputs to the tanks are the result of treatment effects on N_2 fixation. Treatment-specific differences in N inputs from fixation calculated from the TN budgets were corroborated by N_2-fixation measurements (acetylene reduction) made weekly during the month of August (as described in Microcosm Methods), normalized for heterocysts and then scaled to heterocyst abundances over the experiment (Marino, 2001).

The responses of both variables to the Mo addition in the mesocosm experiment were much less than we had expected, especially when considering only the treatment block where grazing was very low (LGr). Orthogonal comparisons (linear contrasts) for the significance of Mo on heterocyst density and TN increases within the LGr treatment block showed a consistent but weak effect (Table 2). Such contrasts are justified because we had predicted that grazing rather than Mo would be the dominant factor in the experiment, based on previous experimental and empirical work on the relationship between N_2-fixing cyanobacteria and Mo availability at sulfate levels greater than 8–10 mM (Cole et al., 1986, 1993; Marino et al., 1990; Marino 2001). While both the heterocyst and the TN data suggest that elevating Mo concentrations in the seawater mesocosms to just above 1 μM ('HMo') may have increased N_2 fixation, the degree of influence of this level of Mo increase is much smaller than our previous kinetic models of sulfate inhibition of Mo uptake had predicted (Cole et al., 1993; Howarth et al., 1999). This result led us to conduct a shorter-term experiment where we varied both sulfate and Mo, and examined the interactions of these on growth and N_2 fixation by a freshwater cyanobacteria assemblage.

Interaction of sulfate and Mo additions in freshwater microcosms

In the freshwater microcosm experiment, sulfate present at 28 mM (the concentration in full-salinity seawater) had a substantial effect on the rate of N_2 fixation: acetylene reduction rates were less than one third of the rate in the Control microcosms (Fig. 3). An analysis of variance showed a highly significant effect of treatment on ethylene production rates ($P = 0.0001$). Planned treatment mean comparisons (orthogonal linear contrasts) showed no significant difference in the response of the Control (no additions) and the Salt Control (chloride addition) treatments ($P = 0.33$, Fig. 3), and a highly significant difference between the addition of chloride salts (Salt Control) and the addition of sulfate ('Sulfate', $P = 0.0001$, Figure 3). The rates of N_2 fixation in the sulfate addition microcosms were 2.1–2.8-fold lower than in the Salt Control treatment, and the sulfate suppression of N_2 fixation is highly significant ($P = 0.0001$, Fig. 3). Note that the reduction in rates of N_2 fixation seen in the sulfate addition treatments is not likely due to an effect of increased osmotic pressure over that of the Salt Control, as the osmotic pressure was higher in the latter. It is also unlikely that the repressive effect of sulfate was due to a difference in ionic strength between the sulfate addition and Control treatments, as the ionic strength of the Sulfate and Salt Control solutions differed by a small amount (0.12 and 0.10 M, respectively), and both were at least an order of magnitude higher than that of the Control treatment (i.e., freshwater ionic strength $\leq 10^{-2}$ M).

The observed inhibitory effect of the sulfate in this experiment was not similarly due to an induced

Table 1. Two factor ANOVA of heterocyst abundance and TN increase in all forms over 1998 mesocosm experiment. Heterocyst data are log-transformed before analysis, as discussed in the text ('Methods'). Heterocyst data were analyzed over two time periods due to an increase of zooplankton in some of the LGr mesocosms during the last two weeks of the experiment (see 'Methods')

		Heterocyst abundance						TN increase		
		Entire experiment (Sept. 1)			through August 17			Entire experiment		
Source of variation	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Grazing	1	0.648	3.332	0.0929	0.693	4.988	0.0453	0.232	27.16	0.0002
Mo	1	0.087	0.447	0.5162	0.037	0.267	0.6149	0.002	0.281	0.6055
Grazing × Mo	1	0.212	1.088	0.3175	0.080	0.574	0.4631	0.028	3.326	0.0932
Error	12	0.194			0.139			0.009		

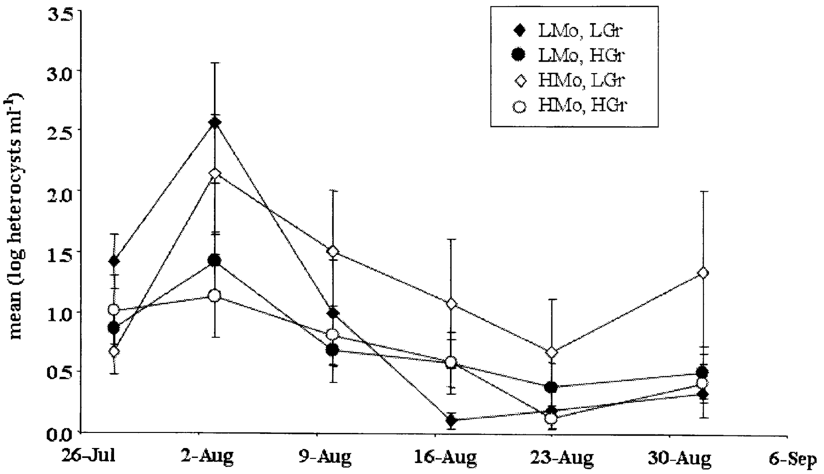

Figure 1. Treatment mean cyanobacteria response (heterocyst abundance) to grazing X Mo treatment matrix over time during the 1998 mesocosm experiment, from first appearance of cyanobacteria until the end of the experiment. Data are log-transformed as described in the text. Error bars are ± 1 SE.

Mg or Ca deficiency, as we purposefully controlled for the potential effect of sulfate complexation on the availabilities of these cations by adding them to the sulfate treatments (and the Salt Control) in constant proportion and concentration. While a portion of the Ca and Mg ions in the sulfate-addition treatments was likely complexed with sulfate in this experiment, as in seawater (10–20%), the availability of these cations in the sulfate and Salt Control treatments was in fact greater than in the freshwater Control, which received no Ca or Mg additions. Measurements of total phytoplankton biomass (as chlorophyll *a*) at the end of the experiment showed a pattern similar to that of the acetylene reduction data, although less extreme, further supporting a specific effect of sulfate on the N-limited phytoplankton community in the microcosms (Table 3).

The addition of molybdate and sulfate ('Sulfate+Mo' and 'Sulfate+10X Mo' treatments) resulted in rates of N_2 fixation that were elevated by 14–25% above those in the Sulfate treatment (Fig. 3). Although the effect of Mo in reversing the repression of N_2 fixation was small relative to the suppression by sulfate, it was significant ($P = 0.013$). Within the Mo-addition treatments, we found that increasing the molybdate addition from seawater levels ('Sulfate+Mo') to 10 times greater than the seawater concentration ('Sulfate+10X Mo') had no further effect on increasing N_2 fixation; in fact the rate appeared to be slightly less than for the treatment with seawater levels of molybdate (Fig. 3;

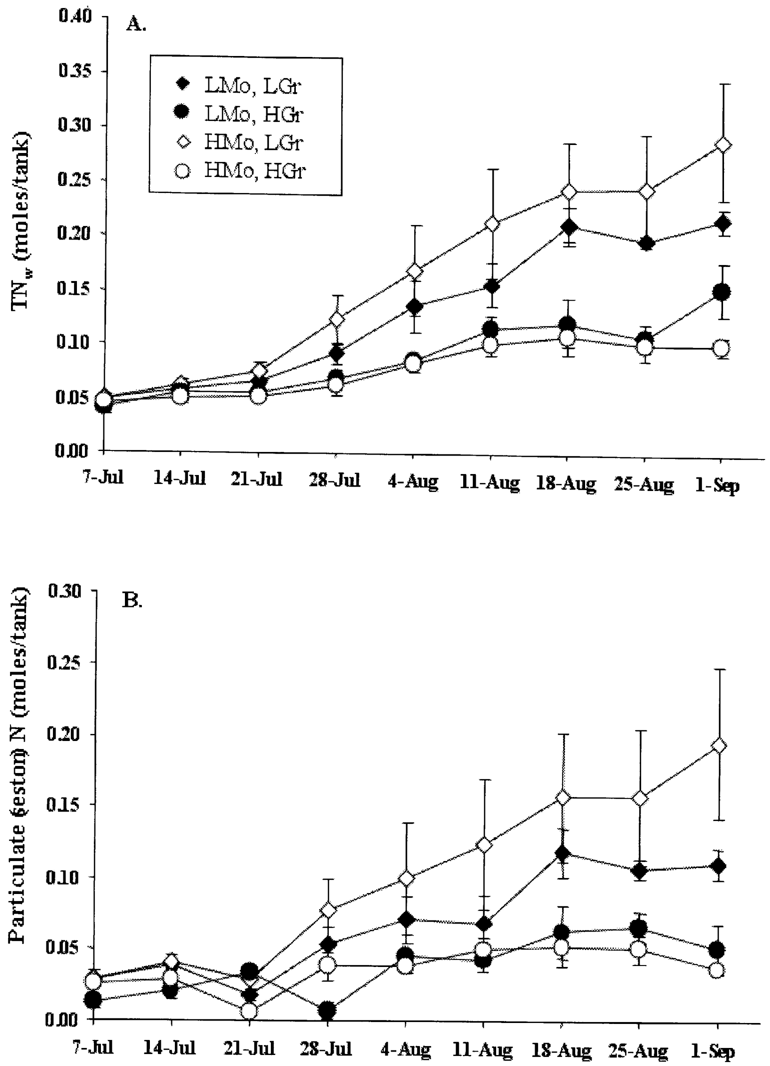

Figure 2. Changes in water-column total N (TN_W; top panel, A) and particulate (seston) N with time (bottom panel, B) during the 1998 mesocosm experiment. Error bars are ± 1 SE.

$P = 0.05$) and was not significantly different from the Sulfate treatment ($P = 0.12$; Fig. 3). Cell abundances in the seawater-level sulfate and Mo-concentration treatment ('Sulfate+Mo') were signficantly lower than that of the freshwater-type sulfate and Mo treatments (controls), as were cyanobacterial growth rates estimated from the cell abundance data over the latter half of the experiment (Table 3). This result is consistent with our original hypothesis that sulfate can inhibit the Mo-requiring process of N_2 fixation in seawater relative to freshwaters (Howarth & Cole, 1985).

The most likely explanation for these results is that the 1400-fold increase in sulfate concentration in the sulfate-addition treatments reduced the availability of molybdate, and that this reduction resulted in lower nitrogenase activity and measured rates of N_2 fixation.

Table 2. Orthogonal linear contrast means comparisons for the effect of Mo within the low grazing (LGr) mesocosm treatment block (df = 1) for analyses where grazing was, as predicted, a significant main effect. Units for means are: heterocysts, weighted mean (log abundace per ml, see text); TN increase, moles per tank; water column TN_W change, moles per tank; water column particulate (seston) N change, mmoles per tank

ANOVA dependent variable	HMo mean	LMo mean	MS	*F*	*P*
Heterocyst abundance	1.285	0.907	0.285	1.466	0.249
Total N increase	0.475	0.366	0.024	2.771	0.122
Water column TN_W change	0.264	0.190	0.011	2.971	0.110
Water column seston N change	167.5	81.5	14766	4.401	0.058

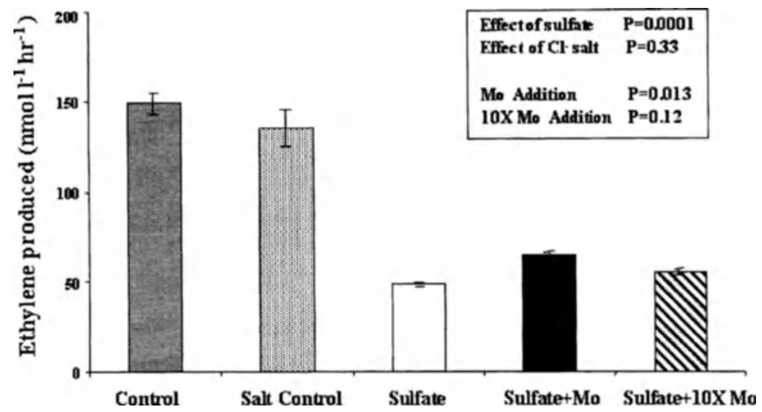

Figure 3. Nitrogen fixation rates for the freshwater microcosm experiment; treatments are as described in the text. N_2 fixation, assayed using the acetylene reduction method, is expressed as nanomoles of ethylene produced per liter of water sample per hour. Error bars are ± 1 SE. See text for further discussion of statistics given.

Table 3. Phytoplankton biomass (chlorophyll), cell densities on the final day of the 1999 pond microcosm experiment, and growth rates estimated from cell densities during the latter half of the experiment. NQ indicates not quantified. All values are means ± 1 SE

Treatment	Chl *a* ($\mu g\ l^{-1}$)	Cells (no. ml^{-1})	Cell Growth Rate (d^{-1})
Control	34.7 ± 0.4	68 899 ± 54	0.32 ± 0.006
Salt Control	36.6 ± 1.8	63 656 ± 2494	0.33 ± 0.024
Sulfate	24.0 ± 1.2	NQ	NQ
Sulfate + Mo	27.2 ± 1.2	36276 ± 2804	0.17 ± 0.013
Sulfate +10X Mo	26.2 ± 0.5	NQ	NQ

While several other environmental and biogeochemical factors besides Mo sufficiency can affect nitrogenase synthesis and N_2 fixation (Bothe, 1982; Van Baalen, 1987; Howarth et al., 1988b; Cole et al., 1993; Vitousek et al., 2002), all conditions other than Mo and sulfate concentration were kept constant across all treatments in the experiment, except for Mg and Ca availabilities, which as noted above were actually increased somewhat in the sulfate-addition treatments.

The mechanism whereby sulfate specifically would have depressed the rate of N_2 fixation is plausible in that it is consistent with the kinetic studies of Cole et al. (1993) and others showing that sulfate depresses the uptake of Mo in N-fixing cyanobacteria, natural phytoplankton assemblages, and heterotrophic bacteria (Elliot & Mortenson, 1975; Cole et al., 1986; Corcuera et al., 1993; Grunden & Shanmugam, 1997). In this experiment, a 20–200-fold elevation of the molybdate concentration in the sulfate-addition treatments partially alleviated the repressive effect of sulfate, although the effect was relatively small. That is, molybdate concentrations up to 10 times higher than seawater, and 200 times that of the environment from which the cyanobacteria were taken did not fully reverse the inhibiting effect of sulfate on N_2 fixation. This finding is consistent with the results from the estuarine mesocosm experiment, and again was not what we had expected based on our earlier Mo kinetic model (Fig. 4).

Reinterpretation of Mo uptake kinetic data

The results of the meso- and microcosm experiments presented here contrast sharply with the responses we predicted based on our previous work (Cole et al., 1993; Howarth et al., 1999; Fig. 4). This earlier work had assumed that the inhibitory effect of sulfate on Mo uptake was reversible if the Mo concentration were increased sufficiently, as is characteristic of competitive inhibition of single enzyme activity. The Howarth et al. (1999) formulation of Mo uptake and inhibition by sulfate (based on the kinetic measurements in Cole et al., 1993), parameterized with the concentrations of Mo and sulfate used in our freshwater microcosm experiments, predicted that Mo availability in the Sulfate+10X Mo treatment would be elevated approximately 5-fold over that of the Sulfate+Mo treatment and 2-fold over that estimated for the Control treatment. Similarly, the Howarth et al. (1999) model predicted that the Mo-addition treatment (HMo) in our estuarine mesocosm experiment should have increased Mo availability approximately 5-fold over the controls for that experiment, yet we detected little if any response in either experiment. These results have led us to re-examine the analysis of the kinetic data at the core of the competitive inhibition model.

The Mo kinetic data published by Cole et al. (1993) are from measurements of molybdate uptake using ^{99}Mo for several taxa of cyanobacteria from pure cultures and the natural phytoplankton community from six freshwater lakes. In that study, we found that molybdate uptake data suitably fit a saturation kinetics model over a range of Mo concentrations that spanned very low freshwater levels up to approximately three times that of seawater. As in earlier studies, Mo uptake was actively mediated (Cole et al., 1986; ter Steeg et al., 1986), and sulfate inhibited the uptake of Mo by phytoplankton at millimolar concentrations ranging from 5% of seawater up to full salinity seawater (Cole et al., 1986). In the Cole et al. (1993) paper, we presented detailed kinetic data for two experiments on the effect of sulfate on molybdate assimilation: one with a pure culture of a freshwater N-fixing cyanobacterium (*Anabaena cylindrica*), and one with the ambient, mixed phytoplankton community of a freshwater lake with N-fixing cyanobacteria (Chodikee Lake). Both systems were actively fixing N_2 at the time of the experiments (Cole et al., 1993). The data were analyzed to determine the kinetic parameters of V_{max} (uptake rate when the enzyme is saturated with the substrate, molybdate) and K_m (the molybdate uptake rate (V) at half saturation) by applying the commonly used Lineweaver-Burk, or double-reciprocal, linear transformation of the Michaelis-Menten equation (Lehninger, 1970). The y-axis ($1/V$) intercept gives the value of $1/V_{max}$ in these plots, and x-axis ($1/[S]$) intercept gives the value of $-1/K_m$, with a slope of K_m/V_{max}.

Here we re-plot the Cole et al. (1993) data in the basic Michaelis-Menten form for the *Anabaena cylindrica* culture and Chodikee Lake experiments (Figs 5-a and 6-a), and then apply a different linear transformation of the Michaelis-Menten equation, plotting the uptake rate (V) as a function of $V/[S]$, where S is the concentration of the substrate (Figures 5-b and 6-b). In the latter plots, known as Eadie-Hofstee plots, the y-axis intercept yields an estimate of V_{max}, and the slope of the line is $-K_m$ (Lehninger, 1970). We use this transformation because it is also a relatively simple way of estimating V_{max} and K_m and, more importantly for the question at hand, because it magnifies departures from linearity that can be difficult to detect using the more common Lineweaver-Burk plots (Hofstee et al., 1959; Lehninger, 1970; Rudolph & Fromm, 1983).

In an Eadie-Hofstee plot, competitive inhibition would be indicated by a series of different lines for different inhibitor (sulfate) concentrations, with each line having the same y-intercept, or maximum rate of Mo uptake (V_{max}) at some saturating level of Mo. Neither the *Anabaena cylindrica* culture (Fig. 5-b) nor the natural lake populations (Fig. 6-b) showed this behavior. For the *Anabaena cylindrica* data of Cole et al. (1993), the data for any given sulfate concentration appear reasonably linear when displayed in an Eadie-Hofstee plot, but the y-intercepts do not converge on a single value of V_{max} (Fig. 5-b). In that experiment, the majority of substrate (Mo) concentrations were relatively low even for freshwaters, with only 3 points at or above 1 nM Mo and the highest at 75% of the seawater concentration (Fig. 5). The range of substrate concentrations used in that experiment limits the detail of $V/[Mo]$ curves at various sulfate concentrations in the region of the plot that approaches the y-axis (Fig. 5-b), making a precise estimate of V_{max} difficult.

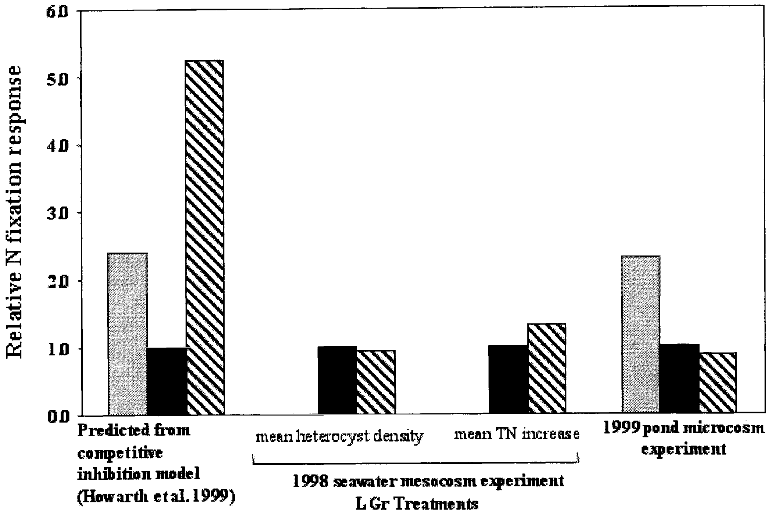

Figure 4. Comparison of relative N-fixation responses at different sulfate and Mo concentrations, as predicted from the model of Howarth et al. (1999), and as observed in the 1998 estuarine mesocosm experiment and the freshwater microcosm experiment presented here. Modeled response is based on an assumed direct relationship between the effect of sulfate inhibition on Mo uptake under typical freshwater and seawater conditions using a competitive inhibiton model (see Equation (1)) and cyanobacterial abundance and N-fixation activity. Gray bars represent typical freshwater sulfate and Mo concentrations (as in Howarth et al., 1999) for the model prediction, and ambient pond sulfate and Mo for the microcosm experiment (see text). Black bars represent seawater-type sulfate and Mo conditions (24–28 mM and 0.1 μM, respectively), corresponding to the LMo mesocosm treatment and the Sulfate+Mo microcosm treatment. Striped bars are seawater sulfate and 1 μM Mo, corresponding to the HMo mesocosm treatment and the Sulfate+10X Mo microcosm treatment. Measured (experimental) or calculated (model) responses are scaled so that seawater-type conditions = 1.

We therefore examined another ^{99}Mo uptake experiment with an *Anabaena cylindrica* culture conducted under similar conditions, but where substrate concentrations covered more of the range between freshwater and full salinity seawater, and extended to 3 times seawater levels (J.J. Cole & others, unpublished data; Fig. 7). Note that Mo uptake rates per unit mass of chlorophyll were higher in this second experiment, as was nitrogenase activity (750 vs 100 nmol ethylene l^{-1} h^{-1}; unpublished data), probably reflecting a difference in the exact portion of the log growth phase of the batch cultures when the experiments were run. These data also appear to follow the Michaelis-Menten model and are reasonably linear when displayed in a Eadie-Hofstee plot, with all lines having R^2 values of 0.9 or higher (Fig. 7-b). Sulfate clearly has an effect on molybdate assimilation, with generally lower molybdate uptake rates at higher sulfate concentrations. Note, however, that again the effect of sulfate does not appear to be one of a competitive inhibitor acting on a single enzyme system: the lines do not converge on one y-intercept ($V_{\max}$), indicating a common maximum uptake rate of Mo at some saturating Mo level, but rather each concentration of sulfate (represented by a single line) has a distinctly different intercept.

At the lowest level of sulfate tested in this second *Anabaena cylindrica* experiment (0.03 mM), the $V_{\max}$ for Mo uptake is fairly low (Fig. 7), perhaps indicating that very low sulfate concentrations can limit the overall metabolic activity of the cyanobacteria. Such limitation is not unreasonable, as sulfur is an element essential in substantial quantities for cell growth, and particularly for synthesis of phycobiliproteins used by cyanobacteria as accessory pigments for photosynthesis and at times as a N storage reserve (Wyman & Fay, 1987; Ortega-Calvo & Stal, 1994). Sulfate starvation has been shown to limit photosynthesis and growth on all N sources, as well as the fixation of N_2 in a non-heterocystous cyanobacterium; sulfate added to 0.3 mm alleviated the deficiency after 2 days (Ortega-Calvo & Stal, 1994).

In the experiments of Cole et al. (1993) with a mixed species natural population of phytoplankton from Chodikee Lake containing actively N-fixing cyanobacteria, an Eadie-Hofstee transformation shows distinctly non-linear behavior at all sulfate levels

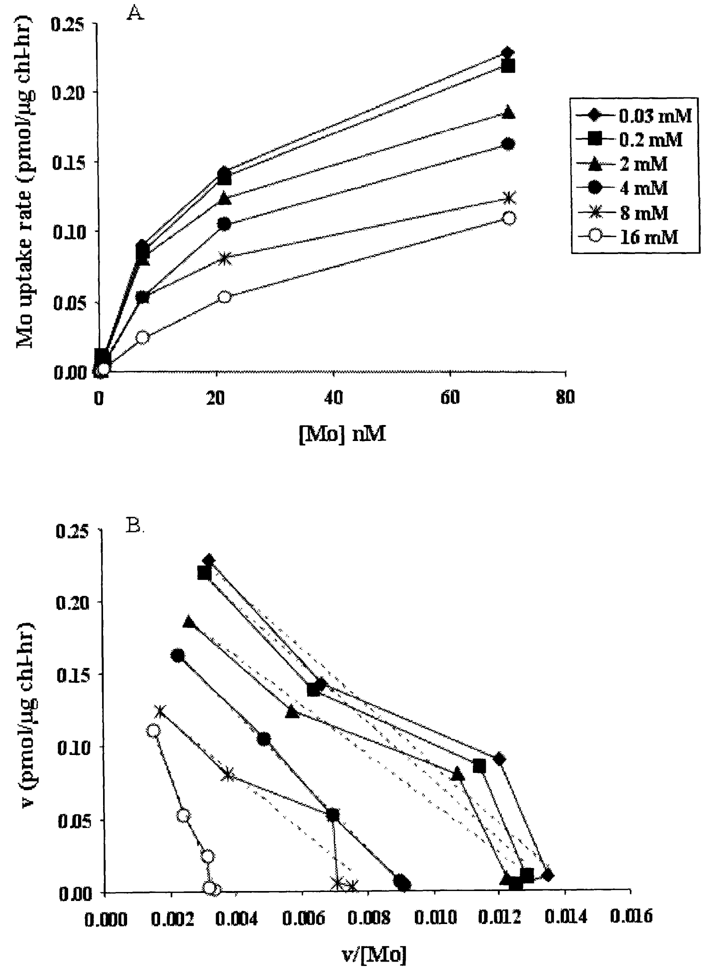

Figure 5. (A) Uptake of molybdate, normalized to chlorophyll, as a function of molybdate concentration for a culture of *Anabaena cylindrica*, in the presence of 6 levels of sulfate (from Cole et al., 1993). (B) Eadie-Hofstee plot of the data in A. R^2 values for linear regressions at each sulfate level are as follows: 0.03 mM = 0.91, 0.2 mM = 0.94, 2 mM = 0.94, 4 mM = 0.99, 8 mM = 0.88, 16 mM = 0.98.

tested (Fig. 6-b). As a result, neither V_{max} nor K_m can be uniquely determined for a given sulfate concentration. This response is perhaps not surprising because, as pointed out by Cole et al. (1993), the uptake parameters measured for such a natural water are averages of the community response and other species of algae in addition to the N-fixing cyanobacteria were present. However, as with the *Anabaena cylindrica* culture experiments, there is still a clear suggestion that sulfate affects V_{max}, with the saturating level of Mo assimilation reached at a lower uptake rate as the sulfate concentration increases from 0.1 to 24 mM (Fig. 6).

The inescapable conclusion of the kinetic data analysis is that sulfate reduces molybdate uptake by natural phytoplankton communities containing N_2-fixing cyanobacteria and pure cultures of common heterocystic N_2-fixers (*Anabaena* spp.), as we had expected based on the strong similarity in stereochemistry. Further, and contrary to our original model of the sulfate-Mo interaction in natural waters, the repressive effect of sulfate is not simply competitive inhibition

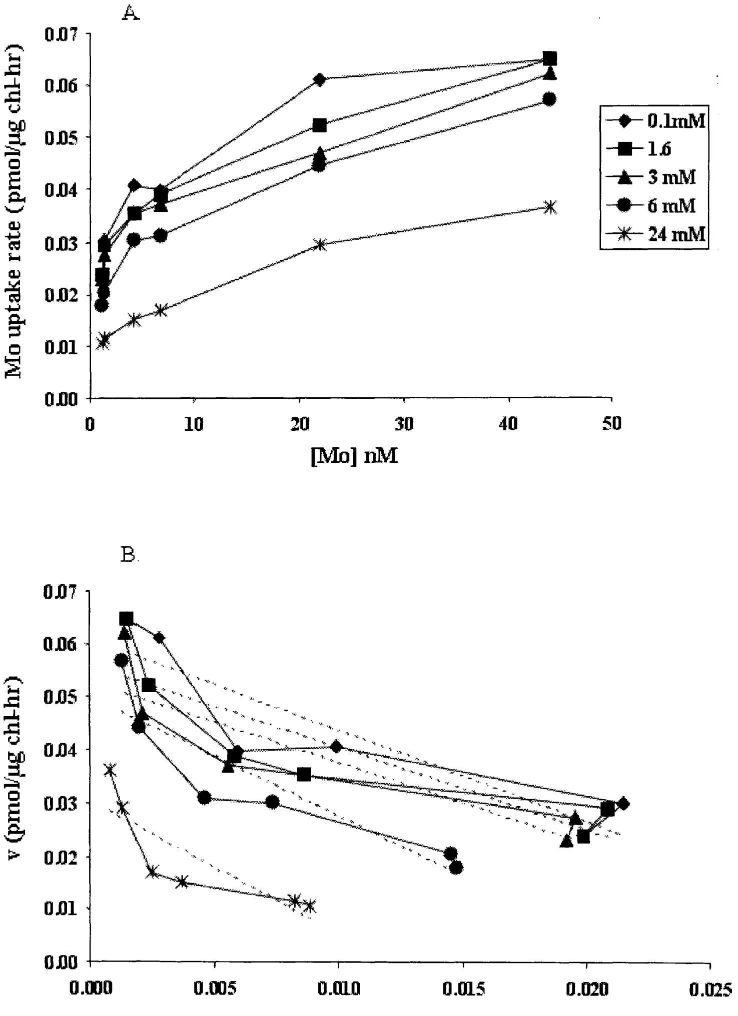

Figure 6. (A) Uptake of molybdate, normalized to chlorophyll, as a function of molybdate concentration for the natural population of eutrophic Chodikee Lake, in the presence of 5 levels of sulfate (from Cole et al., 1993). (B) Eadie-Hofstee plot of the data in A. R^2 values for linear regressions at each sulfate level are as follows: 0.1 mM = 0.83, 1.6 mM = 0.77, 3 mM = 0.77, 6 mM = 0.83, 24 mM = 0.69.

of one enzyme and is not entirely reversible by increasing the concentration of molybdate. That is, it may well not be possible to show experimentally a large increase in Mo assimilation and directly related physiological processes such as increased heterocyst differentiation and N_2 fixation under inhibiting levels of sulfate (mM) by altering the Mo concentration as suggested by the Howarth et al. (1999) model, at least within the environmentally reasonable levels of sulfate and molybdate used in the studies to date. The Mo assimilation data available for cyanobacteria and natural phytoplankton assemblages suggest that Mo uptake is sometimes characterized by a high degree of discrimination between sulfate and molybdate at mM sulfate concentrations, and that the Mo transport system used by an organism tends to be somewhat tuned to be most

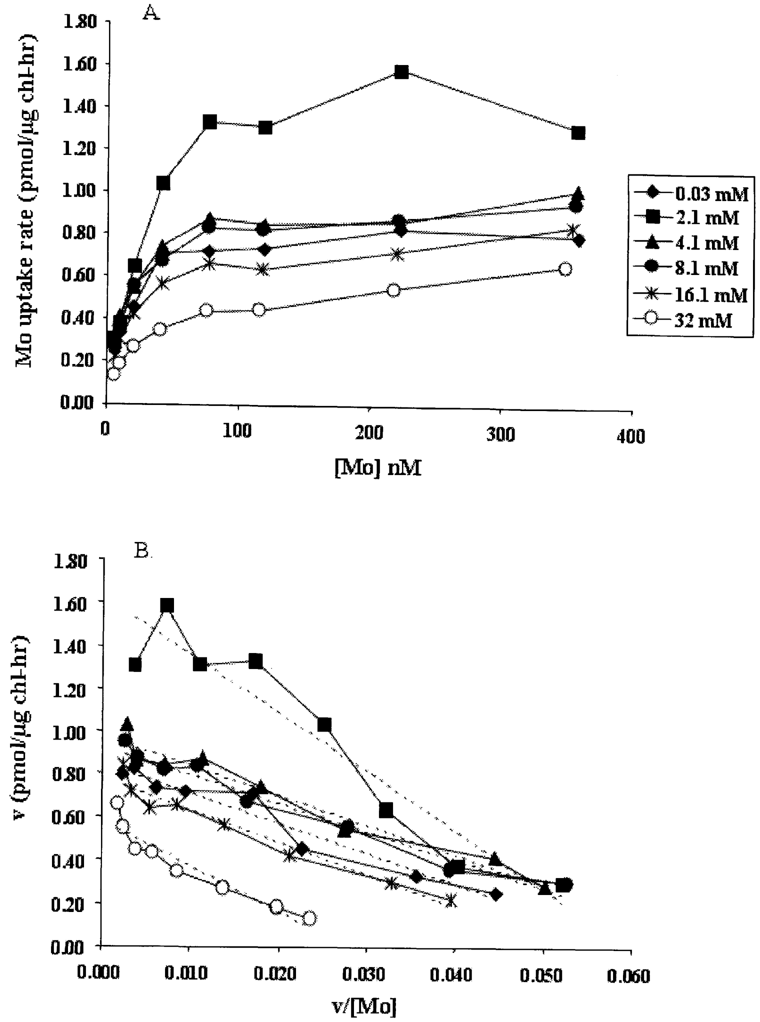

Figure 7. (A) Uptake of molybdate, normalized to chlorophyll, as a function of molybdate concentration for a culture of *Anabaena cylindrica*, in the presence of 6 levels of sulfate (J. J. Cole et al., unpublished data). (B) Eadie-Hofstee plot of the data in A. R^2 values for linear regressions at each sulfate level are as follows: 0.03 mM = 0.94, 2.1 mM = 0.91, 4.1 mM = 0.96, 8.1 mM = 0.97, 16.1 mM = 0.95, 32 mM = 0.90.

effective in the range of environmental conditions (and so Mo availability) typically present (Cole et al., 1986, 1993; ter Steeg et al., 1986).

The combination of the new analyses and experiments presented here with our prior results suggests that Mo uptake may involve two or more enzyme systems, which are activated in response to the ambient environmental sulfate and molybdate concentrations. We hypothesize that when sulfate concentrations are low, a Mo-uptake system that is fast (a high value of $V_{\max}$ and relatively low value of K_m) but not particularly selective for molybdate over sulfate is activated. As the sulfate concentration increases to the millimolar levels typical of brackish and coastal marine waters (approximately 8–28 mM), a non-selective uptake system would lose its ability to assimilate molybdate effectively due to swamping by the stereochemically similar sulfate. The cyanobacteria could then switch to a more selective enzyme system that is better able to distinguish between the two anions and assimilate molybdate much more selectively in the higher sulfate environment. However, the cyanobacteria would pay an energetic price in that this more selective enzyme has a lower maximum uptake level and likely needs a higher ambient concentration of Mo to reach this level; the slower rate of Mo uptake can then limit growth if it is dependent upon N_2 fixation to meet its N need (Howarth et al., 1999). The more specific enzyme may also be more energetically expensive to synthesize or use, further limiting the growth rate of N_2-fixing cyanobacteria. Our hypothesis of multiple Mo-uptake enzymes for cyanobacteria, with varying specificity, is consistent with studies of Mo transport and interaction with sulfate uptake by *E. coli* (Corcuera et al., 1993; Grunden & Shanmugam, 1997) and other heterotrophic bacteria (Maier et al., 1987; Graham & Maier, 1987). The existence of high- and low-affinity transport systems in phytoplankton and other microorganisms for uptake of other essential elements such as Mn, N, and P (as a function of their environmental availability) has also been documented (Carpenter & Guillard, 1971; Medveczky & Rosenberg, 1971; Brown et al., 1978; Nalewajko & Lean, 1980; Sunda & Huntsman, 1986).

Conclusions

It is a somewhat confusing but important distinction that although the absolute concentrations of Mo are high in seawater relative to most freshwaters, the actual availability of Mo in seawater may be much lower than in freshwater. This dichotomy results, at least in part, from the two to three order of magnitude higher concentration of sulfate in seawater coupled with the demonstrated ability of such sulfate levels to inhibit Mo transport. In more saline environments (generally higher molybdate concentration but lower availability than freshwater), the uptake system for Mo needs to be more selective (higher affinity), but this likely requires more energy and so provides a negative feedback to the growth rate of heterocysts and cyanobacterial cells, especially under N-depleted environmental conditions.

The results of our estuarine mesocosm and freshwater microcosm experiments strongly suggest that it may not be possible to alter experimentally the availability of Mo, and so the growth rate of N_2-fixing cyanobacteria in seawater, in a significant manner by adding Mo at environmentally reasonable concentrations (below 10 μM; see Marino, 2001) due to the constraints of the Mo uptake system at high sulfate concentrations. Nonetheless, sulfate clearly has an effect on N_2 fixation and growth of N_2-fixing cyanobacteria, most likely by making Mo less available. In our microcosm experiment with freshwater cyanobacteria, increasing the sulfate concentration to seawater levels (28 mM; Sulfate treatment) depressed N_2 fixation by 63% relative to the rate measured under freshwater conditions. The re-analysis of the ^{99}Mo data for *Anabaena* cultures (Cole et al., 1993) presented here suggests that high sulfate concentrations (16–32 mM) lower Mo availability by 25–65% relative to typical freshwater sulfate levels (Figs 7-b and 8-b). These values are similar to the prediction of the single-enzyme model used in Howarth et al. (1999) that the sulfate concentration in seawater would lower Mo assimilation to a rate that is 42% of that calculated for typical freshwater sulfate and Mo concentrations (Figs 3 and 5). That is, the re-analysis of the Mo-availability portion of the Howarth et al. (1999) model as presented here does not lead to any major change in conclusion concerning the relative difference in availability of Mo at seawater vs. freshwater sulfate levels, or regarding the ecological significance of a 40–60% reduction in cyanobacterial growth rate in estuaries when coupled with grazing pressure (Howarth et al., 1999; Marino et al., 2002). However, the re-analysis does have a profound effect on how one tests the influence of sulfate on Mo uptake across a sulfate concentration gradient.

It seems evident from the analysis and discussion presented here that sulfate at seawater-type concentrations has an inhibitory effect on Mo assimilation and so on the activity of the molybdo-enzymes necessary for the fixation of N_2. This conclusion is consistent with the empirical evidence on Mo distributions in fresh and marine waters as discussed above (Dumont, 1972; Berrang & Grill 1974; Manheim & Landergren, 1978; Howarth et al., 1988b), and with our earlier empirical and experimental results in natural systems (Howarth & Cole, 1985; Marino et al., 1990). However, it is also apparent from this work that the inhibitory effect of sulfate on Mo availability is not completely reversible, as we had expected if the model of competitive inhibition of a single enzyme system previously postulated from our Mo uptake studies was correct (Cole et al., 1986, 1993; Howarth et al., 1999). The controls on Mo transport in planktonic algae and cyanobacteria across a range of sulfate and Mo concentrations in aquatic ecosystems are clearly complex, and a multi-enzyme, mixed kinetics model may better explain the overall data available from several different experimental scales. Although cyanobacteria have had very long time to evolve to the seawater chemistry of sulfate and Mo, the stereochemical similarity of the sulfate and molybdate anions may provide a fundamental constraint under oxic conditions: It simply may not be possible to construct an uptake system that is highly specific for molybdate, and so functions optimally in the presence of high levels of sulfate, without a high cost in terms of Mo uptake (and so growth rate on Mo-requiring N sources) and /or the energetic cost to the organism.

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The contribution of scientific information to the conservation and management of freshwater biodiversity in tropical Asia

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Abstract

Tropical Asia (i.e. the Oriental biogeographic region) is the most densely populated and degraded region on Earth with the highest deforestation rates in the tropics. Flow regulation is a significant threat to riverine biodiversity in the region, and its impacts are combined with overharvesting, pollution and other sources of habitat degradation. In addition to these immediate threats, the potential impacts of exotic species and climate change are difficult to predict. Uncertainty about impact effects arises also from the fact that knowledge of the rich freshwater biodiversity of tropical Asia is incomplete, and up-to-date national or regional inventories are lacking. In part, this reflects taxonomic constraints, and a limited representation of Asian science in the international limnological and conservation literature. A survey of recent (1992–2001) international journals dealing with freshwater ecology and limnology in general, on one hand, and conservation biology on the other, reveal that the representation of scientists based in tropical Asia was extremely low. Scientists from tropical Asia authored fewer than 2% of more than 4500 papers dealing with freshwater biology; 57% of them were published in Hydrobiologia. Less than 0.1% of freshwater biology papers dealt with the conservation of biodiversity in tropical Asian fresh waters. The representation of Asian freshwater science in the conservation biology literature was also poor; 0.6% of 1880 papers surveyed. Such limited dissemination of information reflects a variety of constraints (e.g. manpower, funding, language, and entrenched attitudes), arising from sources both within and outside the region. Even the data that are published are not effectively deployed toward conservation ends. Awareness of some of the more egregious examples of overharvesting (e.g. of river turtles) in the region has increased, but strategies for the protection of riverine biodiversity remain underdeveloped. Where legislation to protect water resources has been put in place, it has been directed towards enhancing human use of water – not biodiversity conservation – and enforcement is weak. Exceptionally, the Chinese government has produced national 'Red Data Books' for endangered freshwater vertebrates, and legislation aimed at protecting species at risk, particularly from overharvesting, are in place. Huge obstacles remain, especially in the management of rivers crossing international boundaries. The Mekong River Commission (MRC) provides an example of a model for an international drainage basin that has made significant progress in establishing appropriate structures and mechanisms for sustainable development in a challenging political landscape. Not all of the Mekong riparian states participate in the MRC, and this will be essential for sustainable management. However, even within national borders, local interests can override drainage-basin perspectives. In many places in Asia, preservation of near-pristine freshwater environments is not a realistic option. Sustaining human livelihoods is an over-riding concern, and recognition of this fact must be built in to biodiversity conservation efforts. This has special implications for the management of exotic species, as the example of fish introductions to the Sepik River in Papua New Guinea shows. Notwithstanding the various factors that constrain publication by scientists in tropical Asia, we must recognise that poor dissemination research results will have consequences for the long-term preservation of the habitats and biodiversity that we study. A change in research strategy that establishes priorities, recognises the inevitability of trade-offs, and includes greater emphasis on engagement and partnerships – as in the MRC – is mandated.

Introduction

What is the state of research on the fresh waters of tropical Asia? Where are we now? Where are we going? These questions need to be addressed – and answered – urgently because the on-going degradation of Asian inland waters will have long-term consequences for biodiversity, human welfare and livelihoods. This paper deals with the contribution of scientific information to the conservation of freshwater biodiversity – a matter that is central to the scientific study and sustainable management of lakes, rivers and wetlands. The implications for humans of success or failure in this regard are evident. They do not arise only as function of the supply of fresh water for drinking and agriculture. Capture fisheries in landlocked Lao PDR, for example, are derived entirely from inland waters, and most of the protein eaten by the Cambodian population is in the form of river fishes harvested from the Mekong and Tonle Sap. Even in Papua New Guinea, which has an extensive coastline, the freshwater catch is greater than marine landings, and more than 80% of the population depends on it (Coates, 1987).

In Bangladesh, as much as one half of the inland catch consists of the anadromous Hilsa Shad (*Tenualosa ilisha* (Hamilton): Clupeidae) although this proportion has been declining. The reduction is due to overfishing and particularly the obstruction of upstream breeding migrations by barrages on the lower Ganges (Loh, 2000); the Hilsa fishery in India has collapsed entirely (Chandra et al., 1990). The danger posed to Hilsa by dams had been pointed out decades earlier (Hora, 1942), and in combination with overfishing and pollution have caused declines in shad species elsewhere in Asia (e.g. Liao et al., 1989; Wang, 1996; Brewer et al., 2001). Maintaining the integrity of riverine ecosystems benefits humans notwithstanding any intrinsic value that may be inherent in species, genes and natural communities. The example of the Hilsa also demonstrates that the transfer from scientific information to conservation and effective management is not automatic, and this has important implications for the practice of our science.

Threats to freshwater biodiversity

There may be no region in the world where the global trends of imperilment of freshwater biodiversity are more conspicuous than in tropical Asia (i.e. the monsoonal lands south of latitude 30 °N or the Oriental biogeographic region). Recent reviews (Dudgeon, 1999, 2000a,b,c,d, 2002a,b) underscore the parlous state of the region's rivers; many lakes have already been degraded irretrievably, and some are little more than giant fishponds. This is not a new conclusion; the manifest imperilment has been apparent for over a decade (e.g. Dudgeon, 1992). Furthermore, Asia is the most densely populated area on Earth, with a high proportion of rural dwellers (more than 70% in India) and some of the world's poorest peoples. Unsurprisingly, much of the landscape is disturbed and degraded; over 60% of it is human dominated (Hannah et al., 1994), and tropical Asia is threatened by the highest relative rates of deforestation and logging in the world (Laurance, 1999; Achard et al., 2002). Even the most recent estimates of forest disturbance (e.g. Achard et al., 2002) do not include the effects of the huge Indonesian forest fires of 1997–1998. Nor do they take account of the post-1997 period of economic depression in Southeast Asia, when the extent of illegal logging and land clearance for agriculture would have increased. The degree of threat has been magnified by a trend towards increasingly frequent and intense fires causing degradation of drainage basins and the remaining area of forest (Taylor et al., 1999).

Threats to the biodiversity of Asian rivers and their associated wetlands include water pollution, salinization, and overharvesting (Dudgeon, 2000a,b, 2002a,b and references therein). Flow regulation, which includes dam-building for hydroelectricity and impoundment of rivers to control floods and provide irrigation water, can have profound effects ranging from alterations in the natural flow regime (changes in current speed, flow volume, water temperature and oxygen concentration) to obstruction of fish breeding migrations. Deforestation within drainage basins causes sedimentation and degradation of lakes and rivers (see, for example, Brewer et al., 2001), and conversion of floodplains and riparian zones to agriculture has detrimental effects on the biota of riverine wetlands. Translocation of native species and exotic or alien introductions may present a further threat to indigenous biodiversity, although views on this matter are divided (e.g. Fernando, 1991). In the longer-term, global climate change will have major effects on flow seasonality and discharge volume of Asian rivers (e.g. Dudgeon, 2000b).

Rates of loss

All inland waters are disproportionately threatened relative to other environments. An extinction rate of 4% per decade for the freshwater fauna of North America has been suggested (Ricciardi & Rasmussen, 1999); this is five times higher than the average rate for terrestrial fauna, and comparable to the projected rate of species loss from tropical rain forest. Qualitative and quantitative data reveal a global trend of declines in populations of freshwater wetland vertebrates over the last 30–40 years; decreases average around 50% but are higher in tropical latitudes (Groombridge & Jenkins, 2000; Loh, 2000). Based on these data, the annual rate of population decline for inland water vertebrates over the period 1970–1999 has been estimated at 2.4% (Balmford et al., 2002). One explanation for such high rates of loss is that the approaches used to conserve biodiversity in terrestrial environments may be counterproductive for the conservation of freshwater systems (Moss, 2000) since they emphasize areas of high quality that can be bounded and protected ('fortress conservation'). This approach will not work for an 'protected' river segment embedded in a large, unprotected drainage basin. The extent of a freshwater system is not defined by the wetted perimeter, but by the catchment from which water and material are drawn.

There is a paucity of unimpacted or pristine water bodies, and lack of reliable historical trend data concerning aquatic fauna in Asia. We are uncertain about total species richness and precise rates of species loss, but the combination of high biodiversity and the magnitude of anthropogenic threats may make Asian inland waters among the most endangered ecosystems on Earth. Monsoonal Asia is host to at least 3500 freshwater fish species, and five Asian countries (Indonesia, India, China and Thailand) are included in the top 10 most species-rich countries in the world for freshwater fish. When world rivers are ranked according to their fish species richness, tropical Asian rivers make up 11 of the 16 top ranks and contain more species than might be expected from the relative global ranking of the extent of their drainage basins (for details, see Dudgeon, 2002a). The region is also exceedingly rich in aquatic invertebrates (e.g. Dudgeon, 1999, 2002a).

The limited information available indicates that population declines, range reductions, and species losses are ongoing (Dudgeon, 1992; Kottelat & Whitten, 1996). In Lao PDR, where disturbance of riparian forest has been less than in the rest of Asia, river birds have been severely impacted by habitat alteration and disturbance; some species have disappeared from large portions of their former range (Thewlis et al., 1998; Duckworth et al., 1999). Freshwater fishes have been severely impacted by overharvesting (Dudgeon, 2002b), especially large migratory species such as the endemic Mekong Giant Catfish (*Pangasianodon gigas* Chevey; IUCN classification = Endangered), and sturgeons (*Acipenser* spp.) and Paddlefish (*Psephurus gladius* (Martens); IUCN classification = Critical) in the Yangtze (Dudgeon, 2000b; Hogan et al., 2001). The Mekong is a relatively unaltered river by Asian standards but, in most instances, the impacts of over-fishing cannot be separated from other stressors such as pollution, flow modification or habitat degradation. In the case of the anadromous Hilsa in Bangladesh, part of the stock decline is attributable to intense fishing pressure on juveniles during downstream migrations.

The status of river turtles exemplifies the grave threats faced by riverine biodiversity in Asia. At least 90 species of freshwater turtles and tortoises occur in Southern Asia (including New Guinea). Of these, 37 were recognized in the 1996 IUCN Red List as Vulnerable (VU), Endangered (EN) or Critically Endangered (CE); a further 18 were Data Deficient (DD) but perceived to be under threat (van Dijk, 2000). The 2000 IUCN Red List (IUCN, 2000) classified the number of species at risk as almost double the 1996 figure (18 CR; 27 EN; 21 VU; 6 DD) (Dudgeon, 2002a). The total increased slightly to 79 species in the 2002 Red List (19 CR; 31 EN; 23 VU; 6 DD) (IUCN, 2002). In other words, approximately 80% of Asian turtles are now at risk and more than half is endangered. In response to this level of endangerment, 23 species of Asian freshwater turtles were added to Appendix II of CITES in order to limit trade in the species most at risk. Their addition reflects the recognition that collection for trade (especially for food) is the major threat to turtles. The main consumers of turtle meat are in East Asia (China, Japan, and Korea) where the meat and shells are considered to have medicinal value. It is likely that exploitation rates increased dramatically after the Chinese currency became convertible in 1989. Imports came initially from Viet Nam and Bangladesh and subsequently from Thailand and Indonesia. As wild stocks declined, these countries began acquiring turtles from neighbouring countries and transshipping them to East Asia. Thus turtles in India, Burma, Lao PDR and Cambodia became subject to intensive collection pressures (van Dijk, 2000).

Political and legislative context for conservation

A host of factors constrains biodiversity conservation Asia. They include the dense human settlement of most drainage basins, a lack of scientific information on the ecology of threatened species and habitats, and widespread apathy about conservation and preservation of non-charismatic organisms. Government commitment to biodiversity conservation is limited in most countries. Economic development and human livelihoods are paramount and justifiable concerns of Asian politicians, reflecting widespread poverty and the mandate of regional governments to improve living standards. Unfortunately, adherence to economic imperatives tends to focus perspectives upon the short-term, and there are few signs that the desirable long-term objective of sustainable development – i.e. that actions undertaken today should not reduce the opportunities of future generations to enjoy ecosystem services – is being achieved.

Legislation that deals with protecting water resources has been put in place to ensure an uncontaminated supply of water for human use. It is not usually directed towards preservation of rare or endangered species and almost never concerned with the maintenance of ecosystem goods and services. A lack of relevant laws is often combined with an inability or unwillingness to enforce those that do exist; for example pollution-control legislation that requires adherence to effluent standards (Dudgeon et al., 2000). An exception to this generalisation is a Cambodian law that forbids the capture, sale and transport of two endangered fishes, the Mekong Giant Catfish and the Giant Carp (*Catlocarpio siamensis* Boulenger), but both species are sold illegally in markets or to fish processing factories (Hogan et al., 2001). Another Mekong endemic, the Smallscale Croaker (*Boesemania microlepis* (Bleeker)) is, in theory, protected under a 1991 Lao PDR Ministry of Agriculture and Forestry Decree that made it illegal to catch them during the spawning season or to sell individuals of the species at any time of the year. Unfortunately, the fish is incorrectly listed in law under the junior synonym of *Pseudosciaena slodado*, but it is doubtful that this is the explanation for the continued widespread sale and illegal fishery of Smallscale Croaker in Lao PDR (Baird et al., 2001). It is also exported to Thailand where it is a highly valued food fish. Severe fishing down of stocks (to 10–20% of previous levels) has stimulated the establishment of Fish Control Zones (FCZ) by local fishers in southern Lao PDR. These encompass deep-water, dry-season spawning habitat for Smallscale Croaker and there is evidence of some stock recovery as a result (Baird et al., 2001). FCZ establishment by villages in Lao PDR has also been used to protect deep pools in the river, which are dry season refuges for large species (such as the Mekong Giant Catfish) and important breeding grounds (Poulsen et al., 2002). The effectiveness of such local community action should be monitored and encouraged by national fisheries authorities.

China lacks a comprehensive law on nature conservation (Xu et al., 1999) but has enacted legislation to protect rare and endangered species in 1989 in the form of the China Wildlife Protection Law (CWPL). There is also a fishery law (dating from 1986) that proscribes fishing of 'rare and precious' aquatic animals (Xu et al., 1999), and fisheries authorities such as the Administrative Commission of the Yangtze River Fishery Resources (ACYRFR) have been established. In addition, national Red Data Books for Chinese freshwater fishes, reptiles and amphibians have been produced (Yue & Chen, 1998; Zhao, 1998). The CWPL it has yet to be fully enforced and is limited by the weakness of laws in neighbouring countries (Li & Li, 1998; Li et al., 2000). China has also established a legislative framework for biodiversity conservation subsequent to becoming a signatory to several international conventions and agreements related to biodiversity, a number of action plans have been initiated (for details, see Xu et al., 1999). Problems with implementation arise from the fact that the natural resource laws and regulations – especially for fisheries – have been formulated from the standpoint of economic value, emphasising utilization rather than protection. Although there have been signs of change in some areas (the ongoing expansion of the remit of the ACYRFR) there is still a perceived conflict between economic development and conservation that allows continued exploitation of biodiversity. Insufficient funding or trained manpower and limited data sharing within China (Xu et al., 1999, 2000) exacerbate the situation. Although there are evident exceptions, much remains to be done in terms of legislative measures to protect freshwater biodiversity in Asia.

Scale, complexity and conflicts of interest

In addition to the lack of political commitment to conservation of biodiversity in Asian inland waters, complications arise from features intrinsic to these environments, and especially to rivers. They are open, directional systems, and elements of their biota range widely using different parts of the habitat at various times during their lives. The Mekong Giant Catfish, and many other river fishes in Asia, undertake migrations during the breeding season. Such movements put fish at risk from stressors or human impacts in various parts of the river at different times; long lived species may be particularly vulnerable. Protection of a particular component of the biota (or habitat) will require control over the upstream drainage network, the surrounding land, and also (in the case of anadromous fishes) downstream reaches. A large-scale approach to conservation, operating on at least the drainage-basin scale, will be essential for migratory fishes but is appropriate also for all freshwater habitats (Moss, 2000; see also 'Rates of loss').

Another significant challenge to the conservation of freshwater biodiversity results from the complexity imposed on these systems by watershed divides and saltwater barriers. In the absence of human disturbance, this results in considerable interdrainage variation in biodiversity and high levels of endemism. This is especially notable among fish assemblages evolved in isolated lakes on islands or mountains and inland plateaux, and these habitats can support numerous endemic species. Examples include Lakes Matano (with a species flock of *Telmatherina* spp.: Telmatherinidae) on Sulawesi (Indonesia), Lake Lanao in the Philippines, and Inlé Lake in Burma where 14 fish species (around half of the lake total) are endemic (Giesen, 1994; Kottelat & Whitten, 1996). Lakes on the neighbouring karstic plateau of Yunnan Province (China) contain a high proportion of endemics, among them a species-flock of at least 14 *Yunnanilus* species (Balitoridae). Cave waters in Yunnan and karstic areas of western Guizhou Province also support several endemic species of *Sinocyclocheilus* (Cyprinidae) (Kottelat & Chu, 1988). In addition to a diverse ichthyofauna that includes endemics, both the Yangtze and Mekong also host endemic species flocks of prosobranch gastropods. Other 'hotspots' include blackwater peat swamps and the streams draining them that are home to assemblages of stenotopic fishes and invertebrates (see Dudgeon, 1999, 2000a,d). The lack of 'substitutability' among habitat units means that protection of one or a few water bodies cannot preserve an entire regional freshwater biota, or even a significant portion of it (Sheldon, 1988). Moreover, because many of the species in a diverse biota are rare and their habits are incompletely known, the tendency for habitat degradation and fragmentation to cause a reduction in the overall number of species is a more predictable outcome than the identities of the affected taxa.

The problems posed by scale and complexity are exacerbated by the fact that fresh waters are subject to multiple uses. The interests of people dwelling in different parts of a drainage basin may be in conflict. A specific problem associated with dam building is that the impacts of the dam are felt locally by the rural riparian communities. An outstanding example is the devastation of artisanal fisheries caused by construction of the Pak Mun Dam (completed in 1994) on the Mekong's largest tributary in Thailand (Roberts, 1993b, 1995, 2001a). Dramatic declines in fisheries were also caused by dam construction on a second tributary, the Theun River in Lao PDR (completed in 1998), despite prior knowledge that it would degrade the aquatic habitat downstream (Usher, 1996). Impacts include obstruction of breeding migrations, conversion of a lotic to a lacustrine environment, periodic dewatering or extreme flow variation downstream, and release of warm, silty, oxygen-poor water from the dam (Roberts, 20001a). By contrast to the local impacts of dams, which tend to be to the detriment of livelihoods and biodiversity, most benefits (cheap electricity and industrial development, flood protection in low-lying areas) are felt some distance away, especially in towns and cities. The pattern of local impacts *versus* distant benefits creates conflicts between rural and urban dwellers. These conflicts tend to be settled in favour of the latter group as they live close to or within centres of political power, but continued acrimonious debate in Thailand suggests that the Pak Mun case may prove to be an exception.

There are signs that the concerns of scientists, conservation groups, other non-government organizations, and – more tellingly – donors about the effects of dams have had some influence of the planning and engineering processes. Nonetheless, discussions about minimum flows needed to maintain ecosystem functions in river reaches downstream of dams have scarcely begun in Asia, and the design of fish ladders and passes suitable for indigenous fishes has received little attention. Where fish ladders have been built, they have been unsuccessful because they follow designs appropriate for salmonids. Few Asian river fishes jump. At Pak Mun Dam, for example, observations suggest that no gravid females of any species can ascend the ladder, and scarcely one quarter of the 258 species in the Mun River can climb it (Roberts, 2001a).

The role of scientists and scientific information

The inland waters of tropical Asia constitute a valuable natural resource, in economic, cultural, aesthetic, scientific and educational terms. They are now being degraded, yet their conservation and management is in the interests of all nations and governments. However, effective conservation and management depends upon the availability of relevant information, and effective legislation. What can scientists contribute that will minimise the constraints on conservation action? The answer depends on whether or not these constraints fall within the purview of science rather than those of, say, politics or economics.

Our ability to quantify the biodiversity crisis in Asian inland waters is limited. This constrains our ability to do anything about it. Basic ecological information on major freshwater ecosystems, such as lowland peatswamp, have yet to be collected and applied to management (Phillips, 1998). Asia is the richest part of the world in terms of specialist river birds, with around half of the total species, yet fewer publications on these animals originate from Asia than from any other region (Ormerod, 1999). We lack robust data on the population status of the vast majority of rare or economically important freshwater species. In fact, species totals for the major rivers of the region appear to be underestimated significantly. For instance, the values for the Kapuas River (250 species) in Groombridge & Jenkins (1998) do not match those of Kottelat & Whitten (1996) for the same river (320 species). Rainboth (1996) estimated that the Mekong drainage may support as many as 1000 fish species, more than twice the total given by earlier workers, while the most recent figure puts total richness in the order of 1700 species (Sverdrup-Jensen, 2002). The unreliability of estimates of species richness in individual river basins makes it virtually certain that national inventories, collections and taxonomic knowledge in Asia are inadequate to document extinctions (e.g. Pethiyagoda, 1994; Kottelat & Whitten, 1996). The situation is exacerbated by the high proportion of rare species in communities of river fishes (Sheldon, 1988) resulting in species being misidentified, or not represented in collections, or listed incorrectly on protected species lists (Kottelat & Whitten, 1996) as in the case of the Smallscale Croaker mentioned above.

A further constraint is that landing statistics for wild-caught river fishes, prawns and so on are in extremely short supply in Asia (FAO, 1999), and some of the figures that are available do not distinguish capture fisheries from aquaculture yields. The result is that population declines are undocumented and thus undetected by fisheries regulatory authorities. On a global scale, Harrison & Stiassny (1999) have classified the freshwater fishes thought to have become extinct over the past 500 years. Rigorous criteria to establish extinction were applied, based on the conservative approach of 'extant unless proven extinct'. They used record 172 putatively extinct species; 59% (102 spp.) of them from Lake Victoria, and around 13 spp. (some with unresolved taxonomic status) from Lake Lanao in the Philippines. While many species of freshwater fishes might be extinct, their results show that very few (only three!) can be shown to be unequivocally extinct; i.e. 'resolved extinctions'. (On the basis of less conservative criteria Ricciardi & Rasmussen (1999) estimated that at least 40 of 1061 species of North American freshwater fishes become extinct during the 20th century.) Harrison & Stiassny (1999) consider that the quality of data from Asia is particularly poor, where only around one quarter of all putative extinctions are well-supported by field work and reliable taxonomic work. There are either not enough data, or the data are too imprecise and incomplete to fully support many of the proposed extinctions. In part, this reflects inadequate field surveys and unrepresentative sampling, so that presence or absence cannot be established unequivocally. Caution is needed in interpreting the limited data available; fish checklists are based more on dated literature or old museum collections than on recent field surveys, and a riddled with errors in species identification (e.g. Pethiyagoda, 1994; Kottelat & Whitten, 1996; see also Roberts, 1993a). Unfortunately, the information for many threatened fish species in the Chinese Red Data book (Yue & Chen, 1998) is based on incomplete or out-dated field surveys. Nevertheless, initial compilation of such inventories is an important contribution since they can always be improved or enhanced once an initial version has been made available.

Even among groups of animals that are taxonomically well known, discoveries are being made in and along Asian rivers. A new species of river bird endemic to the lower Mekong basin – *Motacilla samveasnae* Duckworth & Alström, the Mekong Wagtail – was described in 2001 (Duckworth et al., 2001). Its distribution is restricted making makes it vulnerable to modification of flow and sedimentation patterns (Davidson et al., 2001). Given that species such as the Mekong Wagtail are still being discovered, it follows that additional field surveys of regional biodiversity and the population status of rare species are needed. Enhancement of taxonomic capabilities will need to occur in tandem with this fieldwork. Significantly, the Mekong Wagtail was overlooked initially because it resembled a recognised taxon (Duckworth et al., 2001).

The extent of contribution to the scientific literature

There appears to be paucity of published research on the conservation of freshwater biodiversity, and "... the mainstream conservation community has not given this critical issue the attention that it requires" (Abell, 2002: p. 1435). In this context it is important to determine what contribution have scientists in tropical Asia made to the scientific literature on fresh waters. And, whether much of this literature is relevant or applicable to the conservation of freshwater habitats and their biodiversity.

Methods

For the purposes of this analysis, tropical Asia was defined as above (i.e. the lands south of latitude 30° N). The contents of a sample of international journals dealing with freshwater biology and limnology that had been published during the 10-year period 1992–2001 were examined for papers that had been authored (or co-authored) by workers based in Asia. Nine journals were surveyed: Hydrobiologia, Archiv für Hydrobiologie, Freshwater Biology, Limnology & Oceanography (all 1992–2001), Lakes & Reservoirs (1995–2001 only), Aquatic Conservation (1966–2001), Journal of the North American Benthological Society and Wetlands Ecology & Management (both 1997–2001), Regulated Rivers: Research & Management (1999–2001), and Wetlands (2000–2001 only). Special issues that represented the proceedings of conferences (e.g. the Developments in Hydrobiology series of Hydrobiologia) were excluded. Counts of the total number of articles were adjusted in the case of journals (such as Hydrobiologia and Aquatic Conservation) that publish papers on marine and freshwater systems. In these instances, publications on marine and mangrove ecosystems were excluded from the total counts of papers in a journal.

The proportionate contribution of scientists based in Asia to the sampled scientific literature was calculated by dividing the total number of papers in a journal by the number of papers originating in Asia. These papers were also classified according to country of origin and subject. The subject categorization involved a decision as to whether the paper dealt with biodiversity conservation, interpreted in a general, non-restrictive sense. If it did, the paper was classified further according to habitat (rivers and streams; lakes and reservoirs; marshlands; fishponds and others) and taxon/subject (fishes; other vertebrates; insects; other invertebrates; algae; microbes and protists; macrophytes; other topics).

It could be argued that any scarcity of papers about conservation of Asian biodiversity in journals dealing with limnology and freshwater biology is simply a result of scientists choosing to publish such papers in journals that focus on conservation. To test this hypothesis the papers published in three high-impact biodiversity conservation journals were surveyed: Conservation Biology, Biological Conservation (both 1992–2001) and Biodiversity & Conservation (1997–2001). As above, special issues were excluded as were papers dealing with Asia that were written by persons with institutional affiliations outside the region.

Results (1): the freshwater literature

A total of 4579 papers was included in the survey. Only 75 of them were authored or co-authored by scientists based in tropical Asia; i.e. 1.6% of the total. This percentage figure would be increased slightly if taxonomic research on organisms collected from Asia but undertaken by scientists working elsewhere had been included (an outstanding example would be the former Editor of Hydrobiologia, Prof. Henri Dumont). However, this would have confounded the purpose of the survey. The most striking result was that only four papers focussed upon biodiversity conservation: Dudgeon (1992), Ganasan & Hughes (1998), Singh & Sharma (1998) and Balke (2001); three of them considered biodiversity in the context of assessing environmental impacts. A fifth paper (Kaul, 1995) addressed conservation of water resources in India and a sixth (Xu, 1996) described the 'ecosystem health' of a eutrophic Chinese lake, but neither discussed biodiversity. In short, less than 0.1% of the papers surveyed were related to conservation of freshwater biodiversity in tropical Asia.

Hydrobiologia contained the largest share of papers (37% of all those examined), with 43 articles originating from Asia (i.e. 57% of the Asian total). Articles from Limnology & Oceanography made up

12% of the total surveyed, but the scope of this journal is such that it places emphasis on papers that deal with hydrology, hydrochemistry and plankton, topics that are not generally associated with biodiversity conservation. Nor has Asia as many large lakes as the north-temperate zone. If we exclude Limnology & Oceanography from the survey result, the overall contribution of articles by scientists in Asia is still fewer than 2%, and only a tiny fraction concerns biodiversity conservation. Of the articles originating from tropical Asia, the geographic breakdown was 41% from China (including Hong Kong), 37% from India, and the remainder from Bangladesh, Malaysia, Nepal, Papua New Guinea, Sri Lanka and Thailand. Most dealt with lakes and reservoirs (56%) with the remaining 44% divided equally between lotic habitats and other inland waters. The distribution across taxa showed that most work was undertaken on non-insect invertebrates (mainly zooplankton), which made up 41% of Asian articles. Almost 22% of papers dealt with insects, 14% with algae (including phytoplankton), and 10% with vertebrates (7% was fish); microbes, macrophytes and other topics constituted the remaining 13%. Over 80% of the articles on non-insect invertebrates were published in Hydrobiologia, many of them dealing with zooplankton taxonomy.

Results (2): the conservation biology literature

Of the 1880 papers dealing with conservation biology that were surveyed, 117 (6%) had been authored or co-authored by scientists in monsoonal Asia, and only 12 of them (0.6%) dealt with freshwater biodiversity. None of them appeared in Conservation Biology, which was the most prestigious of the three journals (ISI impact factor 2.783), and a mere 5% of the total number of papers in this journal originated from Asia (17 out of 351). Biodiversity & Conservation (31 Asian papers; 15% of the total articles in that journal) contained two papers dealing with aspects of Asian fresh waters; one on peatswamps (Phillips, 1998) and another describing the trade in (*inter alia*) turtles and amphibians (Li & Li, 1998). Most Asian papers appeared in Biological Conservation (69 papers; 5% of the total in that journal), reflecting the large number of papers (>1300) published during the 10-year survey period. Only 10 of them (14% of Asian papers; 0.7% of all papers in that journal) dealt with a freshwater biodiversity (Datta & Pal, 1993; Ng et al., 1993; Pandey, 1993; Kruuk et al., 1994; Steubing et al., 1994; Shine et al., 1996; Young, 1998; Hussein, 1999; Brewer et al., 2001; Santiapillai & de Silva, 2001), and all concerned vertebrates.

Other lines of evidence

Is the scarcity of papers from tropical Asia revealed by the present survey an artefact of the methods used, such as selection of journals? A survey of the 1990–1999 limnological literature based on a slightly different sample of journals (Burns, 2001), found that 6% of papers in 1990 dealt with species or habitats in tropical latitudes; the proportion in 1999 (7%) was almost unchanged. This suggests that the low representation of papers from the tropics – and hence from tropical Asia – in the international literature is consistent among surveys and over time. Abell (2002) reports that, between 1997 and 2001, around 4% of the papers in Conservation Biology dealt with freshwater species. From the results of the present survey, it is evident that none of them originated from Asia. In fact, only 2% of papers on any subject in Conservation Biology in 2000 and 2001 were from Asia; 68% were from authors in the United States (Meffe, 2002).

Even more surprising is the low submission of manuscripts from scientists in tropical Asia to journals that deal explicitly with tropical habitats and species. Biotropica is the major publication of the Association for Tropical Biology, and the papers are listed on the contents page under the two subheadings of 'Tropical Biology' and 'Tropical Conservation'. Data in the 2002 annual report of the Editor of Biotropica show that, based on the address of the first author, Asian scientists were responsible for an average of only 7% (range 4–11%) of submitted manuscripts between 1996 and 2002 (R.J. Marquis, University of Missouri-St. Louis, pers. comm.).

A telling analysis of the contributions made by Asian scientists is shown by an analysis of fish and aquaculture research in India between 1994 and 1999 (Jayashree & Arunachalam, 2000). This is a subject area that can be considered as applied (*sensu* Denny, 2000), and we might expect a more substantial output than seen for papers on ecology or conservation (see *Financial constraints*). Almost 6% of the global output of papers came from India; 70% of them were published in Indian journals. Few of the papers appeared in journals with a substantial impact factor (many had no impact factor), and government institutions, which responsible for 61% of publications, tended to favour low impact and low visibility journals. Even when the journal impact factor was high (>2.0), however, published papers were not cited at all or, at best, cited infrequently (Jayashree & Arunachalam, 2000). The poor citation may reflect the fact that many researchers in Asia have rather limited access to the scientific literature, notwithstanding the increased availability of on-line resources in some places. It seems that even if an Asian scientist publishes in a high impact journal, the work is not effectively disseminated to those who might wish to make use of the findings. In other words, sometimes information is not accessible in the very region where it is most pertinent (Gopal, 1997).

Content of the Asian literature

Thus far, the focus has been on quantities of information and the number of publications originating from tropical Asia. Qualitative aspects such as applied *versus* basic research, citations and impacts, and so on have not been considered. The Asian freshwater literature may have distinctive or special features not seen elsewhere. Ponniah (2001) reviewed Indian publications on fisheries and aquaculture between 1988 and 1997. They made up 5% of the world publications in these subjects (similar to the 6% reported by Jayashree & Arunachalam, 2000), but instead of dealing with the indigenous fauna many of them concerned exotic species. Almost half (47%) of pollution studies relevant to fisheries science selected tilapias (*Oreochromis* spp.) as model species. Less than 1% used Rohu, *Labeo rohita* (Hamilton), the indigenous cyprinid that featured most frequently in Indian publications. Even for this fish, publications on its ecology and genetics were rare (9% of total publications on Rohu; Ponniah, 2001). The bias towards applied research is evident, as is the lack of attention directed towards research on the biology of indigenous species in their natural environments. These findings support other opinions (e.g. Denny, 2000, 2001) that the emphasis placed on applied research in developing countries may actually reduce the capacity to understand the workings of freshwater systems (see *Financial constraints*).

Another aspect of content is highlighted by Abell (2002) who noted that papers dealing with freshwater species in Conservation Biology were heavily skewed toward the topics of amphibian declines and exotics, particularly the effect of exotics on amphibians (Abell, 2002). Neither topic has received much attention in Asia, where overharvest and habitat alteration are the main threat to anurans (although see Dudgeon & Lau, 1999). This difference is warrants some consideration, as it highlights a difference in attitude between workers in the temperate realm and those in tropical Asia. The issue of exotics in Asian fresh waters is contentious. Some workers have expressed concern over their impacts (e.g. Ng et al., 1993; de Silva & de Silva, 1994; Pethiyagoda, 1994), and exotic fishes occur in more than 90% of the major lakes of Indonesia (Giesen, 1994), but others champion the introduction of certain exotics in particular circumstances.

Fernando (1991) states that contrary to prediction, fish introductions have not caused severe damage to indigenous species except where the exotics were piscivores. For example, circumstantial evidence indicates that the exotic predator *Hypseleotris agilis* Herre (Eleotridae) caused the extinction of members of an endemic species-flock of cyprinids (*Puntius* spp.) in Lake Lanao, the Philippines (Capuli & Froese, 1999). Elsewhere in Asia, however, matters are less clear-cut as it appears that introductions can impact indigenous species through processes other than predation. Three endemic *Yunnanilus* species that occur sympatrically in an endorheic basin on the Yunnan Plateau have been threatened by the introduction of cyprinid competitors (Kottelat & Chu, 1988). Likewise, the palaemonid shrimp *Macrobrachium nipponense* (De Haan) introduced to Dianchi Lake in Yunnan Province competed with an indigenous atyid shrimp *Sinodina* (= *Caridina*) *gregoriana* Kemp dramatically reducing their numbers (Kottelat & Chu, 1988). Endemic *Yunnanilus nigromaculatus* Regan that fed exclusively on atyid shrimps declined as a result of their disappearance. The extinction of this fish – last collected in 1964 – may also reflect interactions with the cyprinid *Pseudorasbora parva* (Temminck & Schlegel) introduced from elsewhere in China, and high pollution loads in Dianchi Lake. *Yunnanilus pleurotaenia* Regan, also a Dianchi Lake endemic, has not been recorded since 1965 (Kottelat & Chu, 1988).

The expansion of inland fisheries in Sri Lanka following the establishment of tilapias, mainly *Oreochromis mossambicus* (Peters), is an example of the beneficial effects of exotics on human livelihoods. The Sri Lankan reservoir fisheries are among the most productive in the world and there is no evidence that the introduction of tilapia has adversely affected indigenous species (Fernando, 1993, 2000). Two native species of *Labeo* have declined in Sri Lankan reservoirs since the 1950s, but this effect has been attributed to stocking of exotic (and non-breeding) major carps rather than to *O. mossambicus* (Fernando, 1993; de Silva & de Silva, 1994). Fernando (1991: 28) concludes: "... the drawbacks of tilapias are relatively minor compared to their contribution to the fisheries in Asia". This view is in stark contrast to the concern expressed over exotics by those – primarily North Americans – writing in Conservation Biology. It may indicate something about the difference in attitudes toward management of inland waters by workers in tropical Asia, where human livelihoods is a paramount consideration in conservation initiatives, and in North America where this dimension is of lesser concern.

Constraints on publication and information dissemination

Financial constraints

Constraints on participation in the peer-reviewed international literature would account for the scarcity of tropical Asian publications on fresh waters and biodiversity conservation. Financial constraints mean that laboratory facilities and equipment are lacking, dated, or poorly maintained; libraries are underfunded; salaries are so meagre that researchers are often obliged to find additional income; and morale or motivation can be low (Williams, 1994; Denny, 2000). Scientists in Europe and North America can be more active in terms of papers published in peer-reviewed international journals because more money has been made available to support them. This also allows the practice of research that does not necessarily return a direct economic benefit. In contrast, when funding is limited there is a perception that basic research is less appropriate than problem-orientated work in response to specific needs. Denny (2000, 2001) believes that funding limitations in developing countries have led to the neglect of basic research and emphasis on applied science, and that a continued focus on topics such as water quality, pollution and aquaculture will erode our understanding of how natural systems work (see also Ponniah, 2001). In such circumstances too, research on taxonomy – upon which knowledge of patterns in biodiversity depend – is likely to be reduced or curtailed entirely. Despite funding disparities, there is no evidence that freshwater science in the temperate realm benefited greatly from an early start; the pioneering work of Sunder Lal Hora in India shows that this is a misconception (e.g. Hora, 1923, 1930).

Language constraints

Another possibility is that many Asian authors experience constraints because of the need to communicate in a language that is, for many, unfamiliar and is almost always English (Williams, 1988). Regional authors may prefer to publish in local journals as these need not be in English. Furthermore they are more readily available to potential readers, and papers dealing with biodiversity will have more relevance to workers within the same biogeographic region. All of these factors may be influential. They give rise to the impression held by some workers (e.g. Williams, 1994) that tropical limnologists tend to publish one-off reports or papers in journals with a restricted local or regional circulation. To test this assumption Boon (1995) analysed 1356 references cited in a volume of contributed papers entitled Conservation and Management of Tropical Freshwaters in Asia and Australia (Dudgeon & Lam, 1994). Only 30% were from widely circulated international journals. The majority (totally 56%) was from regional journals (22%) or reports and theses (25%) or conference proceedings (9%); the remaining 14% cited books. Jayashree & Arunachalam (2000) found the same sort of trend when they surveyed the Indian fisheries literature (see *Other lines of evidence*), but most Indian journals are in English so it seems unlikely that the tendency to publish locally is a result of language constraints.

Participation in learned societies

The key issue here is one of dissemination of information, as conservation of freshwater biodiversity depends upon the communication of scientific information as widely as possible. The evidence suggests that goal is not being achieved. Participation in learned societies provides important opportunities for communication and interaction among scientists. Wishart & Davies (1998) report that almost 85% of the more than 3000 members of the International Association for Theoretical and Applied Limnology (SIL) live in temperate countries; 8% of society membership is from Asia (including the Middle East). Over 1000 members registered for the SIL triennial congress in 1998; only 18 were from tropical Asia (data from Denny, 2000). One reason for the low participation is that major international conferences are typically held in temperate countries, and the financial constraints on research activity mentioned above apply to travel also. A related issue is the tendency for journals that are affiliated to or published by learned societies to impose page charges on publications by non-members; for example, non-members of the American Society of Limnology and Oceanography must pay to have their work published in Limnology & Oceanography. The combination of limited participation in learned societies and financial constraints further restrict the dissemination of scientific information generated in Asia and other tropical countries.

The limited representation of tropical Asian scientists at many international meetings conferences means that they are relatively less effective at disseminating information to their international peers, and are unlikely to be major participants in debates about research methods, or setting research agendas and priorities for freshwater systems. One practical consequence that will arise from this failure to disseminate information is that conservation action in Asia and elsewhere in the topics will be based on the application of experience gained in the temperate realm. It may fail to take account of the important differences that occur between these latitudes (Williams, 1988; Dudgeon et al., 1994; Boon, 1995).

Limited manpower and the need for capacity building

Regardless of the actual proportions of material being published in international journals *versus* local journals and technical reports (the 'grey' literature) or university theses, the present analysis shows that scientists in tropical Asia produce very many fewer papers than researchers in the temperate realm (see also Burns, 2002). The relative output certainly does not match expectations based on relative human population sizes because population size is not a reliable indicator of the number of scientists. China and India, which are the most populous Asian countries, did in fact dominate the Asian freshwater literature producing almost 80% of papers published internationally. However, some countries (e.g. Cambodia, Lao PDR) were not represented at all, including nations with large populations (e.g. Indonesia: >230 million people).

Denny (2001) gives figures showing that the number of scientists and technicians per 1000 people in Southeast Asia (0.2) is one tenth that in Europe and one twentieth of that in the United States. There is an urgent need for capacity building. This is not a new or novel conclusion (e.g. Williams, 1994; Dudgeon et al., 1994), but a shortage of trained manpower inevitably prevents full participation in the international scientific literature, and will therefore constrain conservation efforts. Training alone will certainly not be enough; a single trained limnologist in an under-equipped and under-funded laboratory will have insufficient critical mass to make anything other than, at best, a local difference in the long term. Collaborative frameworks that link workers within Asia and with those outside the region will be needed to share capacity and reduce isolation (for possible initiatives, see Wishart et al., 2000; Denny, 2000, 2001; Amarasinghe et al., 2001).

Are matters likely to improve in the immediate future? The prevailing situation in China and India, at least, does not provide much basis for optimism (Xu et al., 2000; Abidi & Biradar, 2001). For instance, biodiversity research in China is constrained by a lack of funding and trained manpower, limited accessibility of data and some reluctance of institutions within the country to share or exchange information (Xu et al., 2000). The number of graduates in conservation biology produced by Chinese universities is far less than the demand for such professionals (Xu et al., 1999). There is a growing need for – and increasing shortage of – trained scientific manpower in Indian fisheries, with existing training institutions capable of fulfilling only the present demand for fisheries graduates; less than half of the requirement for trained postgraduates can be met currently (Abidi & Biradar, 2001). A shortage of trained manpower may also be a reflection of government and societal attitudes to conservation and environmental management; if they are not seen as priorities, then students may be reluctant to invest in the training needed pursue a career in these areas.

Among the many other problems that constrain national capacity building are inter-institutional rivalry and a lack of cooperation among the various fisheries colleges and universities. The extent of rivalry among institutions (and, presumably, workers) reported by Abidi & Biradar (2001) might go some way towards explaining the lack of citations of Indian papers on fisheries and aquaculture (Jayashree & Arunachalam, 2000; see *Other lines of evidence*). A related problem is academic inbreeding within the 11 State Agricultural Universities in India; almost all of the staff of these institutions are their own graduates. Suggested solutions include enhanced inter-institutional communication and collaborations, and wholesale academic reform (Abidi & Biradar, 2001). Effective reform will be difficult to implement as anyone working in the relatively well-resourced teaching and research institutions of the northern and southern temperate zones will attest.

Neo-colonial science?

The low contribution of scientists based in Asia to the literature on conservation, fresh water biology and tropical ecology may be, in part, a reflection of what Dahdouh-Guebas et al. (2003) refer to as neo-colonial science. They used the 1999 and 2000 Current Contents database to generate a list of all peer-reviewed papers in basic and applied sciences (including biology and environmental science) that concerned research undertaken in the 48 least developed countries as defined by the Organization for Economic Cupertino and Development. They then determined the country of origin of the authors on each paper. The resulting distribution of author countries was surprising: only 27% of 946 basic science papers describing research carried out in a less developed country included authors from a local research institute. In other words, 73% of these papers had been authored or co-authored by workers from 'developed' countries without including collaborators from less developed countries. Where these collaborators had been included, they were first authors in a minority (30%) of papers (Dahdouh-Guebas et al., 2003). A higher figure of 43% of first authors can be derived from data given by Wishart & Davies (1998: Table 4) that were drawn from a survey of 10 freshwater ecology journals (1987–1996). They found that 18% of all surveyed papers reporting research carried out in developing countries were authored solely by a scientist resident in a 'developed' country. This is despite a trend in the limnological literature towards an increase in both the number of authors on each paper, which may improve the chances of acceptance for publication (see Tregenza, 2002), and a rise in the proportion of papers co-authored by scientists in different countries (Burns, 2002; Wishart & Davies, 2002). When manuscripts submitted to Biotropica between 1996 and 2002 are grouped according to study site location, an average of 15% (range 11–18%) had been carried out in Asia. Less than half of them had a local scientist as first author (R.J. Marquis, University of Missouri-St. Louis, pers. comm.).

A number of papers encountered during the present survey of literature dealing with Asian fresh waters did not include scientists based in the region as co-authors. It was not always clear why this had occurred. In some instances, the paper reported on taxonomic work undertaken on museum specimens that had been collected in Asia some months or years earlier. An individual specialist can amass a large collection of research material during a short visit to a country where the biota is incompletely known (i.e. much of Asia) with little or no input from local scientists. It is impossible to determine whether this represents neo-colonial science *sensu* Dahdouh-Guebas et al. (2003) – i.e. science that deliberately and systematically excludes co-authors from certain countries – but the interpretation of the under-representation of co-authorship by scientists from some tropical Asian countries may be less important than the fact itself. It also gives rise to the peculiar situation that researchers in parts of Asia have to learn about aspects of their natural environment through the writings of their counterparts based in the temperate realm. Even if such scholarship is useful and accurate, its mediation through sources based elsewhere make it susceptible to biases, misconceptions and other problems (Williams, 1988). Indeed, Gopal (1997) has argued that tropical limnology is dominated by the studies of workers based in the temperate realm, with the benefits accruing to those scientists, while the direct return to the tropical host institutions is limited. Wishart & Davies (1998) and Denny (2000) have come to rather similar conclusions.

Solutions (1): dealing with 'temperate intellectual hegemony'

Williams (1988, 1994) has made the important point that workers in the tropics should be encouraged more positively to publish in 'international' journals (mainly those produced in the north temperate region), and become less committed to local or regional journals. As mentioned above, the current situation could reflect a constraint arising from the need to communicate in English. In the field of ecology and evolution, manuscripts by native English speakers have a higher acceptance rates in prestigious journals (Tregenza, 2002). There is evidence also that reviewers for international medical journals in the United States might favour manuscripts from compatriots (Link, 1998). Neither tendency would be to the benefit of the majority of scientists based in Asia. In some parts of the tropics, there is little or no intellectual tradition of writing scientific papers (C. Cressa, Universidad Central de Venezuela, pers. comm.). The process of writing is perceived as difficult and unrewarding, and thus few papers are published. More emphasis will need to be placed on the importance of information dissemination during the training of students before this situation can be improved.

Williams (1988) believed that the situation could be reversed if there were changes in attitude towards more sympathetic editorial treatment of English literacy, additional efforts by reviewers and editors in suggesting corrections or changes to manuscripts, and minor changes in journal policy towards the acceptance of purely descriptive papers. This need not mean acceptance of papers of lower scientific value; anyway, there is no evidence that work originating from Asia or elsewhere in the tropics is of poor quality. What is required is an understanding that facilities and working conditions for some authors are very different from those enjoyed by most editors and reviewers for international journals.

One view is that freshwater research undertaken in the tropics is little more than an *ad hoc*, fragmented and derivative area of limnology that is based upon concepts derived in the temperate realm (Williams, 1994). This 'temperate intellectual hegemony' constrains publication because reviewers tend to view manuscripts reporting tropical studies as interesting regional studies first and contributions to the main body of international literature second, whereas studies of temperate waters are viewed in the opposite way. Thus a study of phytoplankton in a Chinese lake may be deemed 'of regional interest only' whereas this would be much less likely to be the judgement passed on a study of phytoplankton in a North American lake. If this perception is widespread, it will inhibit publication and perhaps even submission of papers from Asia to international journals (i.e. those published in the temperate realm). Indeed, the present survey of the representation of Asian limnologists in the international literature suggests that this may be happening. In order to reverse this trend, editors and reviewers based in the temperate realm will need to adopt a more inclusive attitude to manuscripts originating from the tropics.

Solutions (2): an institutional model for collaborative management

A reasonable response to the problems of scale and complexity of freshwater ecosystems (see 'Scale, complexity and conflicts of interest') is to focus conservation strategies upon the entire fauna within a large drainage basin or 'ecoregion' rather than on management of rare species (Sheldon, 1988). This approach makes sense in the context of ongoing or projected large-scale engineering developments on the Mekong and Yangtze (see Dudgeon, 2000b). In such cases, impacts on entire assemblages of species can be anticipated, yet we have little understanding of the ecology of most of them (e.g. Roberts, 1993a). Even if we can agree that ecological common sense mandates conservation strategies formulated and operating at the scale of the drainage basin (or larger), few mechanisms exist to facilitate conservation and management initiatives on this scale. Particular problems arise in the case of rivers (such as the Ganges) that traverse international boundaries, because activities upstream have downstream impacts and consequences that do not always feed back to those upstream. There is significant scope for conflicts of interest: 40% of the global human population lives in the 263 river basins shared by more than one country.

The Mekong River Commission

Multinational organizations are needed to deal with the challenge of *effective* management of large, complex ecosystems that extend over areas differing greatly in sociopolitical environment and government policy towards the natural environment. These are precisely the situations where conflicts of interest arise over resource use. One such organisation is the Mekong River Commission (MRC), an intergovernmental body created in 1995 by an agreement between the governments of Cambodia, Lao PDR, Thailand and Viet Nam. The MRC was created from the Mekong River Committee, itself a modified version of an international organization established by the four riparian states in 1957, that was intended to coordinate water resource development in the lower basin (for more information, see Dudgeon, 1992). The most important of these was a scheme to build a series of dams along the Mekong mainstream south of the Burma-Lao PDR border, which makes up 75% of the total drainage basin. Twelve sites were identified for hydropower dams in 1994, and there is a substantial literature on the possible environmental effects of their construction (see Dudgeon, 1994). The change from Mekong River Committee to MRC in 1995 led to a gradual transformation in attitude and mandate, towards international cooperation " ... in all fields of sustainable development, utilisation, management and conservation of the water and related resources of the Mekong River Basin" (MRC, 2002: 4). This represents a significant move away from a narrow view of river management and the development of one or two major economic opportunities such as hydropower or irrigation, to the broader perspective of integrated sustainable ecosystem development. Most focus is on issues that effect more than one country and include development of 'rules' for water sharing, monitoring the quality of water resources, and supporting joint planning in the context of an overall Basin Development Plan (BDP) initiated in 2002. The BDP is intended " . . . to identify, categorise and prioritise the projects and programmes to be implemented at the basin level . . . " (MRC, 2002: 8) in the key areas of irrigated agriculture; watershed management; fisheries; hydropower; navigation, transport and river works; water-related tourism and recreation; water supply for domestic and industrial use; and flood management. Economic and social issues are to be considered as the BDP is formulated, and national BDP units in each riparian country ensure representation of national interests, agencies and other stakeholders who might be affected by the BDP.

The MRC has a Secretariat based in Phnom Penh. A Council with Ministerial or Cabinet level representation from all members is responsible for overall governance and means that technical and administrative advice tendered by the MRC is heard by government and policy makers. This institutional arrangement gives the MRC the potential to move from meetings and plans to political agreements and joint research and development programmes. While much has been done, it seems likely that the real political commitment will be put to the test within the next five years when decisions made in the context of MRC programmes within the BDP will begin to be implemented.

China and the Mekong River

The MRC seems to have embraced a change in conception as to the benefits of large, mainstream dams. However, not all riparian states belong to the MRC, and full cooperation over collaborative management remains problematic. A substantial portion of the Mekong (known as the Lancang Jiang) flows through China, but China is not a member of the Mekong River Commission (neither is Burma) and has not signed the 1995 agreement. China is an informal 'Dialogue Partner' (= observer) of the MRC, but is unlikely to join if this would obstruct its own plans for the use of the upper Mekong. These are extremely ambitious, including a cascade of huge mainstream dams in Yunnan Province (Chapman & He, 1996; He & Kung, 1998). Two of them, the Manwan Dam (126 m high; 1500 MW generating power) and the Dachaoshan Dam (110 m; 1350 MW) have already been built; work on the much larger Xiaowan Dam (300 m; 3600 MW) began in 2001 and will be completed around 2010 (Anon, 2002). Others such as the Nuozhadu Dam (254 m; 5500 MW) and Jinhong Dam (118 m; 1500 MW) are under development and more are planned but little information is available (Anon, 2002).

The Chinese portion of the Mekong contributes about 20% of the discharge of the Mekong at its mouth (but the majority in Lao PDR and Thailand), and around 50% of the sediment load (He & Kung, 1998; Roberts, 2001). Due to the enormous size of some of the Chinese dams, downstream effects on flows and sediment loads may be substantial. One prediction has it that by 2010 the dams will reduce wet-season discharge and increase dry-season flows by 50% (Chapman & He, 1996), although other estimates project larger changes (Anon, 2002). Consequence will be an 'evening-out' of the peaks and troughs of the natural discharge regime to which the river biota are adapted (for details, see Dudgeon, 2000b). Changes in silt loads due to sedimentation behind dams will have major implications for riverbed erosion and agriculture downstream. The effects will be felt by the lower riparian states that have little to gain from the construction of mainstream dams in China, thereby setting the scene for international conflicts of interest. Possible impacts on fish ecology will be especially important in Lao PDR, where the freshwater capture fishery provides the main source of dietary protein for the human population. Regulation of Mekong flows may lead to substantial loss of biodiversity within China, especially of migratory fishes and large charismatic species such as the predatory carp, *Percocypris retrodorsalis* Cui & Chu (Roberts, 2001b).

In addition to the planned dam array, China also has schemes to make the Mekong navigable from Yunnan Province some 2500 km downstream to the South China Sea. Creation of this navigation channel will require removal of reefs, rapids, sandbars and islands, modification of riverbanks, and regular dredging. The consequences for water retention are unclear, but flood and drought frequency might be affected (Roberts, 2001b). Planning of the initial stages that involve blasting rapids on parts of the Mekong downstream of Yunnan Province where it forms the boundary between Lao PDR and Burma or Thailand was initiated in 2000, and involved the Transportation Ministries of these four countries. Details of the project are confidential, and the MRC has not been invited to participate in planning or impact assessment. While the MRC has begun to move away from espousal of large water development projects that could profoundly affect the ecology of the lower basin, national governments of some riparian states have yet to adopt the same approach. Nonetheless, the existence of the MRC model of what may turn out to be an effective institutional structure for managing international river basins is encouraging.

Solutions (3): conservation and livelihoods – the case of the Sepik River

Many publications dealing with Asian freshwaters concern exotic species but, as mentioned earlier (see 'Content of the Asian literature'), the focus is often utilitarian rather than documentation of the impact of exotics on indigenous biotas. There are certain instances where exotic species have beneficial effects on human livelihoods (e.g. Fernando, 2000), but this view at odds with the overwhelming focus the negative effects of exotic species in the North American literature on conservation biology (see Abel, 2002). Some reevaluation may be in order, as exotic species can be valuable sources of human food in reservoirs and other circumstances. Furthermore the preservation of near-pristine freshwater environments is no longer a realistic option in most of Asia. The introduction of exotic fishes to the Sepik River in Papua New Guinea demonstrates the potential positive effects of selective introductions, and the manner in which scientific research on exotics in Asia can be applied to benefit human livelihoods without significant impacts of biodiversity. It seems to provide a solution to the perceived trade-off between livelihoods and conservation. The Sepik example is relevant also because it shows that introductions need not have negative effects, and illustrates the context in which some research on exotic species in Asia has been undertaken.

The 1100-km long Sepik River (mean discharge 7000 m^3 s^{-1}; Mitchell et al. 1980) is the largest river system in Papua New Guinea in terms of area drained (78 000 km^2). The Sepik River flood-plain fishery has a low yield of around 10% that from similar rivers in comparable latitudes, and catches on a per caput basis are small (Coates, 1985). Fish yields from the hill-stream tributaries are even lower. The low yields reflect, in part, what Gressitt (1982: p. 898) considers to be "... the general poverty of freshwater animals in New Guinea", and the freshwater fish fauna is quite different from elsewhere in Asia (Allen & Coates, 1990). Cyprinidae (barring exotic *Cyprinus carpio* L.) are lacking, and the fauna is made up of diadromous species (Anguillidae, Lutjanidae and Megalopidae), plus permanent inhabitants of freshwater derived from marine ancestors (Ambassidae, Apogonidae, Ariidae, Eleotridae, Hemirhamphidae, Melanotaeniidae and Theraponidae).

The paucity of fishes in the Sepik reflects the recent geological history of northern Papua New Guinea, and the formation of a river basin in what was a recently uplifted (<6000 years ago) intermontane, marine trough (see Löffler, 1977). By comparison, the Fly River, which drains the south of the island, is slightly smaller than the Sepik but supports approximately twice the number of native freshwater fish species (Roberts, 1978; Coates, 1987). Biological constraints on increasing Sepik fishery yields reflect a lack of ecological specializations among the secondarily freshwater fauna (Coates, 1987), with few native species able to exploit the inundated floodplain. It is also unclear how the larger ariid catfish would cope with increased mortality resulting from greater fishing effort.

The Sepik River supports an important subsistence fishery for people who have no access to marine resources (Coates, 1985). Approximately 400 000 people (13% of the population of Papua New Guinea) live in the Sepik catchment which has the highest population density among the island's river systems; more than 65 000 people inhabit the floodplain, and annual rates of are around 2%. Nutritional surveys in Papua New Guinea have indicated a high incidence (>50%) of malnourishment among children, protein deficiency being a major problem. Significantly, protein malnourishment is commonest in highland regions which may reflect the decline in incidence and abundance of native fishes as the river system is ascended (Coates, 1993a). It has been suggested that Sepik River fisheries could be enhanced, with introduction of an appropriate species that would occupy under-utilized or vacant niches proposed as a possible solution (Coates, 1987, 1993a). This is similar to the situation in Sri Lanka, where the introduction of tilapia filled a niche that had been left vacant in the absence of indigenous species adapted to lacustrine conditions (Fernando, 1993, 2000). In the case of the Sepik, the need for conservation of existing species and habitats that may be threatened by exotics must be weighed against the benefits of improved fish stocks and human livelihoods. Enhancement is clearly possible: about half of the fishery yields in the 1980s was exotic *Oreochromis mossambicus* (Coates, 1985). Given that the Sepik fish fauna is distinctive because of what is absent, rather than what is present, there is a possibility that the introduction of exotic species could increase fish production and have minimal impacts on indigenous fishes (Coates, 1993a,b). It should be stressed that capricious introductions of freshwater fishes have occurred in New Guinea, and would continue in the absence of introductions planned in the context of sustainable development.

Fish introductions took place following evaluation of potential benefits and impacts, some preliminary ecological studies (e.g. Dudgeon, 1994), and with adherence to relevant codes of practice (Coates, 1993b). The Food and Agriculture Organization of the United Nations (FAO), with the support of the Papua New Guinea Government, introduced a suite of exotic species mainly under the aegis of the FISHAID project (1993–1997). Fishes were quarantined prior to introduction, and choice of species was based on their potential to fill vacant niches and have minimal impact on indigenous fishes. *Tilapia rendalli* (Boulenger) was the first species imported and stocked in the Sepik. The transfer within New Guinea of the exotics *Trichogaster pectoralis* (Regan) (Belontiidae) and *Osphronemus gouramy* Lacepède (Osphronemidae) was also mooted but not followed up on a large scale. *Puntius gonionotus* (Bleeker) was the second species stocked in the lower course of the Sepik, while *Barbodes* (= *Acrossocheilus*) *hexagonolepis* (McClelland), *Schizothorax richardsonii* (Gray) and *Tor putitora* (Hamilton) (all Cyprinidae) were introduced in fast flowing stony tributaries at various altitudes. The characids *Piaractus brachypomus* (Cuvier) (= *Colossoma bidens*) and *Prochilodus argenteus* Agissiz (= *P. marggravii*) have also been stocked in floodplain lakes and the lowland reaches of the Sepik. Introduction of the cyprinid *Sinilabeo* (= *Labeo*) *dero* (Hamilton) from India or Nepal has been proposed but not yet undertaken (D. Bartley, FAO, pers. comm.).

Concluding remarks

Conservation of freshwater biodiversity is hamstrung by a lack of information. There are no global estimates for rates of change in the extent of freshwater habitats or for overall changes in their condition. There is, however, a widespread perception that habitat loss and conversion is occurring apace in the Asian tropics (e.g. Foote et al., 1996; Gopal & Junk, 2000), with floodplain wetlands being especially affected. Quantification of these processes and of population trends in threatened and 'indicator' or 'sentinel' species are needed. Information alone is not enough. A wider appreciation of the value of freshwater biodiversity will be essential to ensure its long-term preservation. This depends on realistic economic valuation of the ecosystem goods and services provided by fresh waters (e.g. Constanza et al., 1997; Balmford et al., 2002; Patterson, 2002). However, there is a paucity of empirical data on the question of the yield of goods and services from retaining habitats in a relatively undisturbed condition versus that obtained when they are converted for human use (Balmford et al., 2002).

While more information is desirable, a shortage of research funding and facilities over much of Asia constrains generation of new information. The prevailing economic depression has exacerbated an already unsatisfactory situation. Moreover, even at the best of times, conservation biologists, ecologists and taxonomists do not enjoy priority allocation of funds. We are unlikely to obtain all of the information needed to understand the ecology of all (or the major) component species of the biota of Asian fresh waters in the foreseeable future. We can continue to complain about a shortage of data, but it may be that there is sufficient information available now to allow a 'generally correct' diagnosis, especially if anecdotal or informal observations (e.g. from artisanal fishers) and components of the grey literature are taken into account. Any delay in necessary action that results from attempts to gather more information is unlikely to serve conservation ends. We need to apply what we know now, even if that means the use of approximations and rules of thumb in our efforts to protect threatened habitats and their biota. Note that this does not mean the slavish application of lessons learned from research undertaken in the temperate realm.

A lack of information is not only a function of absolute shortage, but can also be caused by ineffective dissemination or communication of what we know already. The fate of the Indian Hilsa fishery and the examples of the Pak Mun Dam and Nam Theun River demonstrate that even when scientific knowledge is available and can be brought to bear upon development issues, there is no automatic translation of such information into political or legislative action. This suggests that while a paucity of research funding and information constrain conservation efforts in Asia, applying information effectively remains *the* major challenge. The present situation can be summarised as one in which scientists complain that policy makers do not read what they write, while policy makers complain that scientists do not write anything they can read. If we cannot address this mismatch successfully, commitment by governments to the conservation of freshwater ecosystems will be unattainable, and our research will have little influence in a world where vested commercial interests and a disinterest in nature hold sway. Partnerships with organizations such as the MRC, which have the institutional structures to influence governments, will be needed; some of the operational criteria for successful collaboration have been identified by Amarasinghe et al. (2001). In order to ensure that we make available the most useful information for environmental management, we must also identify priorities and formulate action plans – steps that the MRC has begun to take in the context of its BDP.

Scientists in tropical Asia must position themselves better so as to achieve the ends of knowledge generation, dissemination, and its effective application in biodiversity conservation. We must not only publish widely; we must also ensure that our research findings are translated into political, legislative and management action. This may require us to discard our attachment to the notion of the disinterested scholar and broaden our traditional practice of 'science of discovery' to include involvement in a 'science of engagement' (Meffe, 2001) to communicate the results and implications of our work. Care must be taken: there is a trade-off between dispassionate science and environmental advocacy, and we risk being perceived as biased providers of information if the balance shifts too far from objectivity and empiricism. We will also need to make adjustments to what we regard as acceptable forms of ecosystem management. The manifest benefits to human livelihoods derived from exotic species in Sri Lanka and Papua New Guinea indicates that, under circumstances, the maintenance of pristine ecosystems is neither the most desirable goal nor, in the populous, degraded landscapes of Asia, is it even achievable. If we are to develop effective conservation and management strategies, we will need to accept that there will be a trade-off between species preservation and human use of ecosystem goods and services. The latter cannot be prevented (nor, given the level of poverty in Asia, should it) and thus attempts to manage ecosystems that focus solely on maximizing biodiversity will fail. A compromise position of management for ecosystem functioning rather than preservation of every species will provide a better basis for biodiversity conservation in the long term (Moss, 2000).

Well-managed and preserved inland waters are more likely to result from adequate and widely disseminated information, as in the temperate realm. Insufficient or poorly disseminated information will result in bad management, degradation of tropical freshwater ecosystems and a loss of biodiversity. In short, "... lack of information will further impoverish those who lack it" (Williams, 1994: p. 359).

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Lake-based climate reconstruction in Africa: progress and challenges

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Abstract

Lake sediments are and will continue to be the principal source of information on the climate history of tropical Africa. However, unequivocal interpretation of the various sedimentological, biological, and geochemical climate-proxy data extracted from lake sediments with respect to past variations in temperature, rainfall, and wind is an extremely complex and challenging exercise. Outstanding problems are: (1) the inherent conflict between a lake's sensitivity to climate change (its ability to respond to and record relatively modest, short-lived climatic anomalies) and its persistence as an archive of climate change (the probability that it survived the most arid events without desiccation or erosion, allowing it to preserve a continuous record of climate history); (2) the scarcity of annually laminated sediment records, which in other regions can provide superior chronological precision to lake-based climate reconstructions; (3) lack of a quantitative (sometimes even qualitative) mechanistic understanding of the chain of cause and effect linking sedimentary climate-proxy indicators to particular climatic variables; and (4) lack of a proxy indicator for past temperature changes unaffected by simultaneous changes in moisture balance. Clearly, a climate-proxy record with high stratigraphic resolution does not represent a high-resolution record of past climate change without demonstration that the sedimentary archive is continuous and undisturbed; that the lake system responds to climate variability at the appropriate time scale; and that any threshold effects in the relationship between the proxy indicator and climate are accounted for. Calibration and validation of climate-proxy indicators is tantamount to establishing accurate reconstructions, but in Africa historical validation of proxy indicators is handicapped by the scarcity of long-term lake-monitoring data. The reliability of lake-based climate reconstructions is enhanced when inferences derived from several proxy indicators (sedimentological, biological, or geochemical), that each have an independent mechanistic link to climate, show a high level of coherence. Given the scarcity of annually-resolved sediment records in tropical Africa, we may have to accept the limitations of ^{210}Pb- and ^{14}C-based chronologies when evaluating the synchrony of reconstructed climate events between sites and regions; however, careful site selection and detailed lithostratigraphic analyses can go a long way to optimise depth-age models and reduce uncertainty in the timing of past climate changes.

Introduction

An important part of the paleoclimate research enterprise today has shifted attention from the long-term dynamics of Quaternary ice ages and interglacials to the comparatively modest, short-term, and regionally specific natural climate variability that is more directly relevant to human society in the context of global climate change. Improved understanding of this inter-annual to century-scale climate variability requires a global network of accurate, trustworthy climate reconstructions with which climate modellers can test and validate the computer models developed for long-term climate prediction. Accordingly, much emphasis is placed on natural climate archives with high, preferably annual, resolution and time control. Special attention also goes to the tropics, for which the current paucity of relevant paleoclimate data contrasts with its prominent role as the heat engine of the global climate system.

Africa is the quintessential tropical continent. Unfortunately, reconstructing the climate history of tropical Africa over the last few thousand years, and thus the environmental background for its complex precolonial cultural history, is hampered by the scarcity of instrumental and documentary records from before the colonial period (Nicholson, 2001) and by the limited potential of traditional high-resolution climate-proxy archives such as tree rings and ice cores. Very few African trees develop distinct annual growth rings, those that do rarely grow older than 100 years, and dead logs or old construction timber is rarely preserved long enough to be of any use in extending the historical instrumental record (Dunbar & Cole, 1999). Ice cores from Mt. Kilimanjaro have now provided a unique window on Holocene climate and atmospheric composition in equatorial Africa (Thompson et al., 2002), but the reconstruction is handicapped by the lack of an independent chronology for the inferred climate events (Gasse, 2002). Further, the known distribution of high-quality speleothems is still largely limited to Africa's extra-tropical north and south. Compared to these three types of natural climate archive, sedimentary climate records accumulating on the bottom of Africa's many climate-sensitive lakes have great potential to document the continent's patterns of past climate change, with respect to both geographical and temporal coverage.

This paper presents an overview of current research methods available for lake-based climate reconstruction in tropical Africa, with some emphasis on biological climate-proxy indicators and the aspects of lake hydrology and sedimentation that affect their stratigraphic distribution in sediment cores. No attempt is made to review current knowledge about the Holocene climate history of tropical Africa; for this the reader is referred to Gasse (2000) and the relevant chapters in Batterbee et al. (2003). Neither do I review and evaluate state-of-the-art statistical methods in biological paleolimnology, which is the subject of several excellent publications (Birks, 1998; Birks et al., forthcoming). Rather, this contribution represents a personal view of notable recent advances and unresolved problems in lake-based climate reconstruction in tropical Africa. To illustrate such unresolved problems, reference is often made to research in which I have been personally involved, if only because of greater awareness of the hidden weaknesses in my own results than in the work of others in the field.

African lake sediments as archives of Holocene climate variability

Lakes are excellent sensors of environmental change, and sediments accumulating on the bottom of suitable climate-sensitive lakes can provide continuous records of past climate variability with high (inter-annual to decadal scale) temporal resolution (Battarbee, 2000). However, in Africa it is necessary to use the conditional tense here, because the very sensitivity of the continent's many hydrologically-closed lake basins to relatively modest, short-term rainfall variability (or, more precisely, the balance between precipitation and evaporation) also makes them prone to drying out completely, resulting in truncation or partial destruction of the high-resolution climate archive that has been accumulating (Verschuren, 1999a). Hydroclimatic fluctuations in tropical Africa over the past 10 000 years were so dramatic (Gasse, 2000) that most lakes studied thus far which were sensitive enough to record clear evidence of climatic variability within the wet early Holocene have dried out repeatedly during the dry late Holocene; and lakes that survived the most arid episodes of the late Holocene had been less sensitive, hydrologically open systems during the early Holocene. Indeed, most African lakes where a documented 20^{th}-century history of significant lake-level or salinity fluctuation provides opportunity to calibrate and validate their sedimentary climate-proxy record with appropriate temporal resolution do not have a continuous sediment record covering the last 2000 years, let alone the entire Holocene. Consequently, in African lake-based paleoclimate studies, site selection is crucial.

The second major issue is chronology. Proper understanding of the mechanisms of global climate variability at decadal to century time scales critically depends on establishing coherent regional pictures of climate history involving many study sites, and on the ability to compare this history with independently constructed and dated records of the climate drivers that are directly or indirectly responsible for the observed patterns of past climate change. Finely laminated lake-sediment records with annual signal resolution and time control (so-called varved records) are naturally preferred for this purpose, but in tropical Africa such varved records are extremely rare, because the seasonal cycle (the succession of dry and wet seasons, and the timing of deep mixing events) is too complex, or not strong enough, to consistently generate the annual packets of distinct sediment laminae that can give a sediment record superior chronological precision.

The third complicating issue involves the often uncertain relationship between a sedimentary climate-proxy indicator (sedimentological, geochemical or biological) and the primary climatic variables of temperature, rainfall, and wind. In contrast to hydrologically stable study lakes in north-temperate regions, where a range of sedimentary climate proxies can be reasonably assumed to be mostly controlled by temperature variations, a reliable proxy indicator for past temperature change in Africa (and other tropical regions) is still lacking. For example, whereas the oxygen stable-isotope ratio of fossil-diatom silica in northern European lakes appears to be mainly under temperature control (Shemesh et al., 2001), its promise as a temperature indicator for tropical regions was diminished by the finding that even in a high-altitude setting (4300–4600 m) on Mt. Kenya its Holocene signals are better explained by moisture-balance changes (Barker et al., 2001) than by temperature-induced fractionation (Rietti-Shati et al., 1998). Because of the dramatic hydroclimatic changes in Africa during the Holocene, all sedimentary climate-proxy indicators currently in use at least partly reflect moisture-balance variations expressed via changes in lake level, water chemistry and isotopic composition, water-column stratification, and/or sedimentation dynamics. And as mentioned above, without exception the strength of their signatures is inversely related to the probability that the sediment column is a continuous archive of climate history. Lack of an independent proxy indicator for temperature also complicates interpretation of lake-level fluctuations in terms of rainfall variability (Verschuren, 2003). Lake-level change reflects variation in the balance of rainfall and evaporation over the drainage basin, and is thus at least partly controlled by temperature. This temperature effect on lake level is most pronounced in closed-basin amplifier lakes with large drainage basins, because of the dependence of river inflow on basin-wide evapotranspiration (e.g., Vallet-Coulomb et al., 2001). In the large African Rift lakes with comparatively smaller drainage basins, wind speed may have a stronger influence on overall evaporation than temperature (Lehman, 2002).

Lake-based reconstruction of Holocene climate variability in Africa

At glacial-interglacial time scales, the recent climate history of inter-tropical Africa is divided into a fairly wet late Glacial and early Holocene (~15,000–5500 cal. yrs BP; the so-called African Humid Period) caused by strengthening of the equatorial westerlies and the Indian Ocean monsoon system during the Northern Hemisphere insolation maximum, and mostly drier mid- and late-Holocene climatic regimes (deMenocal et al., 2000). Both periods were punctuated by severe dry spells lasting several 100s of years, centered at 12 400, 8200, 6600, and 4000 years ago (Gasse, 2000). The first of these droughts corresponds to the Younger Dryas period in the North Atlantic region, and re-establishment of wetter conditions at 11 500–10 800 years ago coincides with the Pre-Boreal warming of northern temperate regions which marks the onset of the Holocene there (Verschuren et al., 2003). Most probably also the later droughts have mechanistic links to millennium-scale climate variability in the North Atlantic region (Gupta et al., 2003) but their timing has not been sufficiently constrained to explore this in detail. In southern Africa up to Lake Malawi (~10° S), a wet Late-Glacial period appears to have given way to a relatively dry early Holocene followed by a wetter mid-Holocene (Gasse, 2000; Scott & Lee-Thorp, 2003), but with scant evidence of the millennium-scale droughts that occurred in northern inter-tropical Africa.

To date, the only nearly continuous African climate-proxy records spanning the entire Holocene with decade-scale resolution are two fossil-diatom records from Lake Victoria (Stager et al., 1997; Stager & Majewski, 1997; Stager et al., in press, Fig. 1), and one record of biogenic-silica accumulation in Lake Malawi (Johnson et al., 2002, 2003); a third fossil-diatom record from Lake Victoria (Stager & Johnson, 2000) has century-scale resolution, but suffers from an apparent mid-Holocene discontinuity. Lake Victoria has overflowed into the Nile for the past 13 000 years (Talbot et al., 2000), hence sedimentation conditions in deep-water areas (the central basin is 68 m deep) might be expected to have been relatively stable throughout the Holocene. However, ^{210}Pb-dating of recent deep-water sediments (Verschuren et al., 1998) confirms predictions based on wave theory that fine-grained sediments across the entire offshore lake bottom are periodically (once in several decades) subject to erosion and re-deposition,

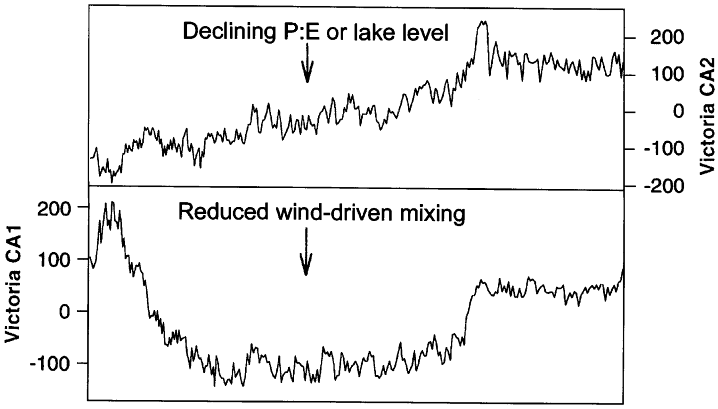

Figure 1. Climate-driven changes in the physical limnology of Lake Victoria (East Africa) over the past 11 000 years inferred from variability in fossil-diatom species assemblages. CA1 and CA2 refer to sample scores along the 1st and 2nd principal Correspondence Analysis ordination axes, summarising the variability in fossil-diatom species composition; P:E refers to the balance of precipitation and evaporation. From Stager & Mayewski (1997).

due to wave turbulence during exceptionally violent storms. This sediment disturbance effectively limits the realised time resolution of Lake Victoria's offshore climate-proxy record to centuries, rather than decades. Higher-frequency signals evident in the offshore fossil diatom record may result from the alternating burying and re-suspension of the surface deposits in which differential diatom preservation occurs, rather than reflecting decade-scale ecological or climatic variability. Site-specificity of sedimentation conditions and the associated taphonomic processes is evident in poor correlation of inferred climate events between the fossil-diatom records from a 30-m deep channel between offshore islands (Stager et al., 1997), the middle of the lake (Stager & Johnson, 2000), and a small protected bay along its northern shore (Stager et al., in press), beyond the broad millennium-scale climate trends shared by all three records.

Pilkington Bay, only 12 m deep today, has probably produced the highest-quality climate-proxy record from Lake Victoria because wind shelter promoted quiet sedimentation dynamics, and proximity of the core site to near-shore benthic habitat translated even modest lake-level fluctuations during the past 1000 years into clear interpretable changes in the fossil diatom flora (Stager et al., in press). Less clear, however, is whether relationships between lake level and benthic diatom abundance in the sub-recent sediment record can be extrapolated to the mid- and early Holocene, when local water depth may have been ~30–36 m due to the combination of high lake level (raised strandlines of assumed early-Holocene age stand 12–18 m above present lake level; Stager et al., 2002) combined with 6 meter less sediment filling the bay. Even if Holocene lake-level fluctuations can be quantified, a direct diatom-climate link at short time scales would seem tenuous because the hydrologically open condition of Lake Victoria mutes its lake-level response to short-term moisture-balance changes, and because a significant portion of presumed mid-Holocene lake-level lowering may be due to the down-cutting of its outflow rather than the transition to a drier climate.

This observation does not reduce the significance of the Lake Victoria fossil-diatom records; rather, it illustrates the notion that a high-resolution climate-proxy record can not uncritically be taken to represent a high-resolution record of past climate change. Indeed, the first purpose of high-resolution stratigraphical analyses is to produce a level of data redundancy that, as the paleo-ecological equivalent to replicate data series, gives credence to the most prominent, and relatively long-term, climate signals present. High-resolution climatic interpretation requires demonstration that the proxy record is continuous and undisturbed; that the lake system responds to climate variability at the appropriate time scale; and that the complex relationships are understood between a buried climate-proxy indicator (e.g., fossil diatom species composition; oxygen-isotope ratio in authigenic carbonate) and its original form (e.g., diatom community composition and annual production; oxygen-isotope ratio of dissolved inorganic carbon), between the proxy indicator and lake hydrology, and between lake hydrology and climate.

African lake-based climate-proxy records that cover the full Holocene with century-scale resolution and time control include a multi-indicator reconstruction (geochemistry, diatoms, and ostracod trace elements) from Lake Tigalmamine in the Atlas Mountains of Morocco (Lamb et al., 1995) and a diatom-based salinity reconstruction from Lake Abiyata in Ethiopia (Chalié & Gasse, 2002). The latter study carries particular interest, because it involves a highly climate-sensitive closed-basin amplifier lake in the semi-arid Ethiopian Rift Valley, and because the paleolimnological studies are part of a wider research programme that includes long-term weather monitoring and hydrological modelling (e.g., Vallet-Coulomb et al., 2001), the kind of process-oriented supporting studies which may eventually allow one to read the record of lake history as a true climate record. However, given the inferred shallowness of Lake Abiyata for much of the past 5000 years (Chalié & Gasse, 2002), it is possible that phases of (near-) complete desiccation created one or more century-scale sedimentary hiatuses that the ^{14}C-based sediment chronology is unable to resolve. Consequently, despite understanding of contemporary lake-climate relationships, correlation of the relatively short-lived climate events revealed in this record with those from other African sites and extra-tropical regions may be difficult.

Selecting study sites for lake-based climate reconstruction

As indicated above, lakes in stable hydrological settings are not typically selected for high-resolution climate reconstruction in Africa, except when the targeted climate-proxy indicator is external to the lake system (e.g., wind-blown dust or glacier advance and retreat) or when the main interest is in long-term vegetation dynamics rather than climate variability per se. Climate-sensitive lakes include hydrologically closed lakes, where water output only occurs through evaporation; hydrologically open lakes where surface or subsurface outflow is low compared to evaporation; and amplifier lakes, where any stabilising effect of a permanent outflow is overridden by highly variable river inflows from a large and well-drained catchment. Several hundred such climate-sensitive lakes occur in the parts of Uganda, Kenya, Ethiopia, and Tanzania which together form the vast sub-humid and semi-arid regions of the East African Plateau. Still, finding lakes with just the right balance of climatic sensitivity (their potential to respond to and record clear signals of short-term rainfall variability) and longevity (their potential to preserve these signals in an uninterrupted sediment sequence) is difficult. Selection of suitable study sites starts with an assessment of basin topography, lake morphometry, and mean and peak annual wind speeds and directions. Together these factors permit application of wave theory (Håkanson & Jansson, 1983) to predict the dominant processes of sediment distribution (Hilton, 1985; Fig. 2) and determine if local sedimentation conditions allow undisturbed, continuous accumulation of a climate-proxy record (Dearing, 1997). Field-monitoring data or equivalent documentary evidence (for example, temporal sequences of aerial photographs and satellite images) on the magnitude of seasonal and inter-annual lake-level fluctuations relative to current lake depth permit educated guesses about the probability that today's favourable sedimentation conditions persisted throughout the period of interest (Verschuren, 1999a). These predictions of the integrity of a lake's climate-proxy record can be tested, by lithostratigraphic analyses and high-resolution ^{210}Pb-dating of short sediment cores representing lake response to climate change during the past ~150 years.

Given that high-resolution climate-proxy records are so easily compromised by decade- to century-scale cryptic hiatuses which (mostly ^{14}C-based) sediment chronologies are unable to resolve, one might be tempted to minimise the risk for such hiatuses by selecting among Africa's many crater lakes that are sheltered against wind-driven turbulence by a high crater rim and are deep enough to tolerate substantial lake-level fluctuations without sedimentation being interrupted or disturbed. However, the steep bottom slopes in these lakes bring increased risk of sediment slumping (Hilton, 1985), so that the integrity of the climate-proxy record is affected by interbedded turbidites. Steep bottom topography also means that appreciable lake-level change is not accompanied by significant changes in lake surface area or volume, thus weakening the signals of most biological and geochemical climate-proxy indicators (e.g., Barker et al., 2000). The main advantage wind-sheltered crater lakes have over larger, more exposed tectonic and floodplain lakes is that favourable sedimentation conditions can

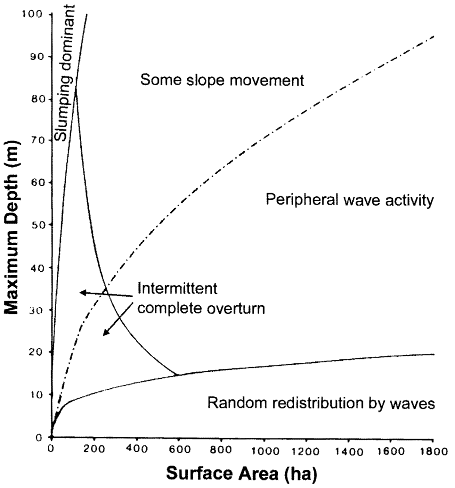

Figure 2. Relationship between lake depth, lake surface area as a measure of effective wind fetch, and prevalence of the four main processes of sediment distribution. From Hilton (1985).

be maintained in very shallow water depths, allowing greater relative amplitudes of lake-level change to be registered intact (Verschuren, 1999a). Hence crater lakes of intermediate depth, which combine adequate wind shelter with gentle bottom topography, appear to be the preferred sites for high-resolution climate reconstruction from a sedimentological standpoint. A potential weakness of crater lakes as climate recorders compared to large tectonic lakes is that their local hydrogeological setting may add complexity to the link between lake level and climate, a problem which must be circumvented by parallel studies of several crater lakes within crater-lake districts.

A unique category of climate recorders are the crater basins confluent with or hydrologically connected to river-fed tectonic lakes, which combine a wind-sheltered sedimentation regime with the climatic sensitivity of an amplifier lake. The best-known example is Crescent Island Crater in Lake Naivasha (Kenya; Verschuren et al., 2000a); others are Lake Sonachi near Lake Naivasha (Verschuren, 1999b), and several unexplored peripheral basins of Lake Turkana (Kenya). The Kibengo crater basin in Lake George (Uganda; J. Russell et al., unpublished data) also provides a locally favourable sedimentation environment, but its hydrological response to rainfall variability is muted by the fact that the Lake George-Lake Edward system is hydrologically open.

Key problems of chronology

Many African lakes deposit finely laminated sediments. Testifying to the absence of significant sediment disturbance (either physical or by burrowing organisms), finely laminated sediment records are invaluable to climate reconstruction because of the superior temporal resolution they can provide. However, truly annual laminations have so far only been demonstrated (through parallel varve counting and ^{210}Pb-dating) in Lake Malawi (Johnson et al., 2001), Lake Hora in Ethiopia (Lamb et al., 2002), and Lake Bosumtwi in Ghana (J. Overpeck et al., unpublished data). In Lake Malawi, varved sediments interrupted by a few homogenites extend from the present to ~AD 1300; down-core the lamination desintegrates into alternating varved, banded, and massive sediments. A second section of apparent varves covers the period ~6500–9000 cal. yr BP, but discrepancy with bracketing ^{14}C dates suggests that up to 25% of the annual couplets there may be unclear or missing (Barry, 2001). Often, when sections of distinct annual lamination do occur in African lakes, they are usually too short (10–50 years) to significantly improve ^{14}C-based age models. Since most lakes suitable for varve formation and preservation have now been surveyed, a distinct possibility exists that tropical Africa will never yield a full Holocene lake-based climate record with annual time control.

Lake-based chronologies of Holocene climate and hydrological change in Africa will thus continue to rely mostly on a combination of ^{210}Pb- and ^{14}C-dating. This entails several problems. First, the most spectacular records of millennium-scale dry spells during the early Holocene (Gasse, 2000) are based on ^{14}C-dating of materials recovered from ancient shorelines and exposed lacustrine sections, limiting their dating resolution and precision. These dry spells have yet to be clearly documented in a continuous lake-sediment sequence supported by a well-constrained AMS^{14}C-chronology. The high-resolution Holocene ice-core record of Mt. Kilimanjaro (Thompson et al., 2002) displays exquisite detail at decadal to millennium time scales, but climatic inferences are weakened by the assumption that the observed oxygen-isotope variability is entirely due to temperature variation (contra Rozanski et al., 1993; see also Barker et al., 2001), and because the inferred timing of events has not been not absolutely dated but based on correlation with ^{14}C- and U/Th dated climate-proxy records both nearby (Lake Naivasha, Kenya) and far away (Soreq Cave, eastern Mediterranean). Consequently, detailed comparison of these millennium-scale events on the African continent with records of Holocene monsoon variability from Oman (Neff et al., 2001) and the Arabian Sea (Gupta et al., 2003) may remain pending for some time. Second, in large African lakes such as Victoria and Malawi, which can be expected to accumulate a continuous sediment record (but see section 3 above), ^{14}C dating is complicated by the scarcity of large terrestrial plant macrofossils at offshore coring sites. Such fossils tend to yield more trustworthy sediment ages because terrestrial plants obtain their starting ^{14}C concentration from atmospheric CO_2. The alternative, measuring the ^{14}C of aquatic algae (as preserved in bulk organic matter) tends to over-estimate true sediment age because aquatic algae obtain their starting ^{14}C concentration from dissolved organic carbon, which in closed-basin lakes with long residence times often has a reduced ^{14}C/^{12}C ratio relative to the atmosphere. In large lakes, where sediments may go through several cycles of resuspension and redeposition before reaching their final burying site offshore, dating bulk organic matter can also over-estimate true sediment age because of contamination with old organic materials (for example, pollen) which are refractive enough to have survived these redeposition cycles intact (Russell et al., in press). The age offsets due to reservoir effects can be corrected by parallel ^{14}C- and ^{210}Pb-dating of pre-1945 sediments. Ideally this is supplemented by parallel ^{14}C-dating on bulk organic matter and terrestrial plant macrofossils at intervals further back in time, to confirm that the lake's reservoir age remained constant throughout the period of the reconstruction, but in large lakes this is rarely an option. In the case of old-carbon contamination, age offsets are also site-specific within a lake, and require ^{14}C age correction for each coring site separately.

Given these complications, the preferred strategy to obtain reliable sediment chronologies is to: (1) select lakes that are small enough to bury adequate quantities of large terrestrial plant fossils at mid-lake coring sites; (2) obtain ^{14}C dates at intervals approaching the 2σ counting error on each date (± 100 years); (3) carefully select which individual dates can be included in the age model, based on understanding of the evolution of local sedimentation dynamics as reflected in lithostratigraphy; and (4) if feasible, increase the precision of the age model further by wiggle-matching sediment ages against the atmospheric ^{14}C calibration curve (Stuiver et al., 1998). This ^{14}C wiggle-matching procedure (van Geel & Mook, 1989) has proved a powerful tool to improve time control in Holocene peat deposits (e.g., Speranza et al., 2000), but so far it has not been applied to African lake sediments. Besides the high cost of close-interval ^{14}C-dating, at least two other complications can be envisioned. First, in peat deposits the dated material is certain to represent the time of peat formation, as the peat mosses themselves make up most of the peat matrix. By contrast, burial of terrestrial plant remains in offshore lake sediments may involve significant delays, due to retention in soils, peripheral swamps, or nearshore sediments. Sturdy plant remains, such as pieces of wood and sedge rhizome, can survive several decades or even centuries of such temporary storage and the transport to their final burial site offshore. For example, Verschuren (2001) excluded three out-of-sequence ^{14}C dates that were 150–300 year older than the expected ages based on a polynomial regression of historical marker horizons, ^{210}Pb-ages, and ten other ^{14}C dates covering the 1100-year history of Lake Naivasha (Kenya). In this particular case, exclusion was justified because all three dates were measured on single pieces of wood or sedge, whereas most of the others were measured on charred grass fragments; and all three were extracted from a single lowstand horizon during which reworking of older littoral deposits is most likely. When pre-burial storage lasts decades rather than centuries, identification of out-of-sequence dates is nearly impossible. Variation in retention times before permanent burial then simply increases the noise in age-depth relationships, with the result that the short-lived ^{14}C-plateaux and reversals in the ^{14}C-calibration curve, which are the time anchors in ^{14}C-wiggle matching, are no longer evident in the sediment chronology despite better-than-century dating resolution. One feasible solution to this problem is to date only those terrestrial plant macrofossils that are likely to be destroyed by prolonged aerial exposure or in the process of sediment reworking, such as brittle leaves of trees and marsh plants, or fragments of grass charred by bush fires. When such plant remains are found in offshore sediments, they most likely reflect direct deposition from the air. A second complication for ^{14}C wiggle-matching in lake records is the requirement that sedimentation rate be relatively uniform at multi-decadal time scales; large sedimentation-rate changes would create spurious ^{14}C plateaux and ramps.

When ^{14}C-wiggle matching is not possible, detailed correlation of high-resolution climate-proxy records, and evaluation of possible leads and lags between them in the expression of specific climatic anomalies, requires that age models at least capture the distortions of the age-depth relationship caused by the changes in sediment-accumulation rate that usually accompany lake-level fluctuations. Without such correction, for example, the duration of a lowstand episode can be either over- or underestimated depending on the severity of the drawdown relative to the lake's critical depth of sediment accumulation (Verschuren, 1999a). When accumulation is continuous throughout the lowstand, accumulation rates tend to increase with falling lake level (see section 7 below); however, when water depth becomes less than the critical depth of sediment accumulation, sediment erosion and redistribution results in little net sediment accumulation during the lowstand (Verschuren, 1999a). Given that some African lakes have been documented to experience up to six-fold changes in mid-lake sediment-accumulation rate within a decade (Verschuren, 1999b; Fig. 6), full correction may prove elusive. Similarly, while a lake desiccation lasting for several centuries typically leaves clear stratigraphical signatures and can be accounted for in an age model, decade-scale hiatuses due to non-deposition or erosion at low lake level may be difficult to recognise because mixing of unconsolidated muds deposited before and after the lowstand obliterates the evidence that the climate-proxy record has been truncated (Verschuren, 1999a).

In these circumstances, demonstrating leads and lags between sub-century scale climatic anomalies across a region becomes challenging. One possibility is to restrict high-resolution climatic inferences to those sections of the individual proxy records with a high probability of continuity and relatively stable sedimentation rates. A coherent picture of high-frequency climate variability throughout the Holocene will then ultimately require combination of early-, mid-, and late-Holocene records from different sites.

Salinity inference as indicator for climate-driven hydrological change

Quantitative paleoclimatology in Africa with biological proxy indicators is currently limited to diatom-based inference of lake-water conductivity (salinity), pH, and ionic composition (Gasse et al., 1995), and chironomid-based salinity inference (Verschuren et al., 2000a; in press). The statistical performance of the African diatom-based salinity-inference model is better than the chironomid-based model, because of more frequent species turnover along the salinity gradient (i.e., average tolerance ranges of diatom species are narrower), but in saline lakes or during saline phases in lake history chironomid-based inferences are potentially more reliable because their chitinous remains are less prone to diagenesis than diatoms (species composition of fossil diatom assemblages being affected by differential dissolution; Barker et al., 1990).

A more fundamental problem with salinity inference, however, is that the relationship between salinity change and climate forcing is indirect and complex (Gasse et al., 1997), involving memory effects of previous hydrological history on current salinity (Langbein, 1961; Barton et al., 1987) and salinity regulation via groundwater seepage (e.g., Telford et al., 1999). Even when a case can be made that long-term salinity variation has been proportional to the climatic fluctuations causing it, the relationship is non-linear and characterised by threshold effects. Apparently, when combined rainfall, river inflow and groundwater seepage fail to keep a tropical lake fresh (below $\sim$1500 μS/cm), perpetual high temperatures and a pronounced local hydrological deficit move it very quickly to true salt-lake conditions ($>$6000 μS/cm) and a shift to specialised fauna and flora. This is attested to by the scarcity of modern African lakes with intermediate salinities (Talling & Talling, 1966; Wood & Talling, 1988); one notable exception is Lake Turkana (3500 μS/cm; Kolding, 1992) the only hydrologically-closed basin among the large African Rift lakes. In fossil records from high-sedimentation-rate environments (e.g., Verschuren et al., 2000a; Fig. 3) it is evident in very sudden transitions between freshwater and salt-lake species assemblages, unduly suggesting very sudden transitions between wet and arid climate regimes. A possible corollary of this observation is that more gradual salinity trends inferred from other fossil records may often be due to post-depositional mixing of freshwater and saline species assemblages, through bioturbation or wind-induced sediment reworking.

Given the complex relationship between lake-water salinity and climate, quantitative salinity reconstruction should ideally be accompanied by site-specific and time-dependent modelling which can transform the inferred trajectories of past salinity change into histories of lake-volume (and thus water-balance) change (e.g., Vallet-Coulomb et al., 2001). Unfortunately, the local meteorological and hydrolo-

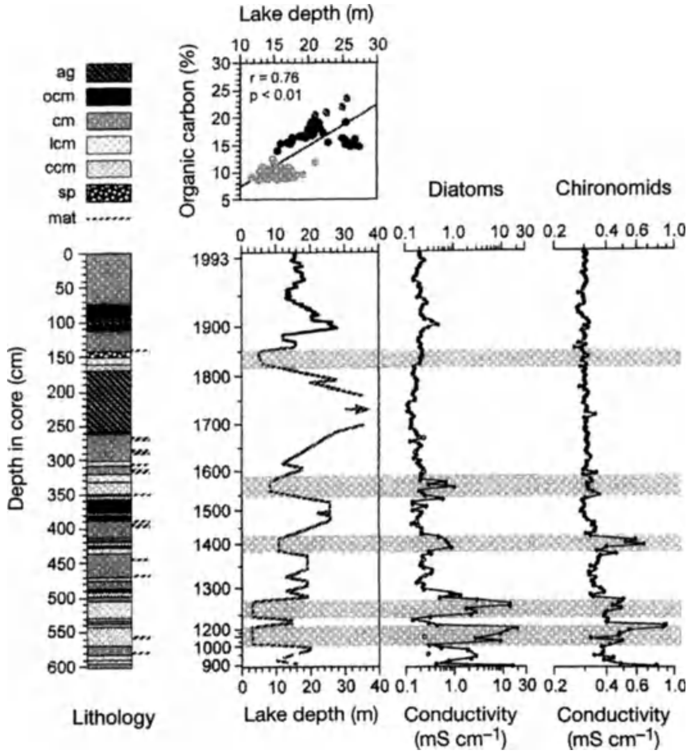

Figure 3. Lake-level and salinity fluctuations in Crescent Island Crater, Lake Naivasha (Kenya) over the past 1100 years reconstructed from sediment texture and organic-matter content, and from fossil diatom and chironomid species composition. Sediment types include algal gyttja (ag), organic clayey mud (ocm), clayey mud (cm), low-organic clayey mud (lcm), calcareous clayey mud (ccm), and silty peat (sp); the relationship between organic-carbon content and lake depth is based on the historical record, AD 1883–1993. Modified from Verschuren et al. (2000a).

gical data required for such modelling are available for only very few African lakes. When quantitative modelling is not an option, salinity reconstructions are validated by comparing the upper part of proxy-indicator records with independent time series of historical data, and using the match between the two as a guide to assess the validity of inferred patterns further downcore. Such comparisons involve direct correlation with instrumental weather data and drought indices (e.g., Laird et al., 1996), or with climatically controlled limnological variables (e.g., Fritz, 1990; Legesse et al., 2002), including historical salinity data.

One particular series of studies (Verschuren, 1999a–b; Verschuren et al., 1999a–b, 2000a) investigated the biological effects of climate-driven lake-level change in the Lake Naivasha system (Kenya), a complex of four distinct lake basins that because of their hydrological interconnectedness have a common recorded lake-level history spanning the last 120 years. In the three basins for which a diatom-based salinity reconstruction is now available, inferred salinity matches the main patterns displayed by historical instrumental data. The main significance of these studies, however, is their illustration of the complexity of chemical and biological response to climate-driven lake-level change at decadal time scales, and how the signatures of this response in the sediment record is affected by sedimentation dynamics and taphonomy. For example, although reconstructed salinity and lake-level changes in Lake Oloidien displayed the expected inverse correlation, this relationship was modulated by apparent delayed dilution of dissolved salts following modest lake-level rise in the late 1950s–1960s (Fig. 4). Given tight sediment chronology, Verschuren

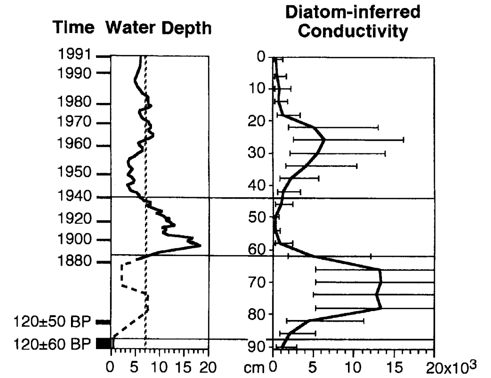

Figure 4. Measured lake-level fluctuations and diatom-inferred salinity variations in Lake Oloidien (Kenya) over the past 120 years (~AD 1870–1993). Due to its dependence on nearby Lake Naivasha for freshwater input, freshening is rapid when transgression results in broad confluence (the 1890s rise), but slow when transgression only increases seepage inflow (1950s–1960s rise). From Verschuren et al. (1999b).

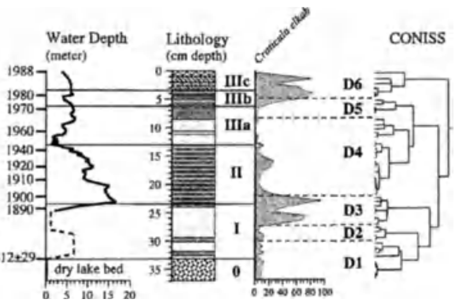

Figure 5. Measured lake-level fluctuations, sediment lithology, CONISS fossil-diatom zonation, and stratigraphic distribution of the dominant shallow-water diatom *Craticula elkab*. The boundary between diatom zones D3 and D4 post-dates the lithostratigraphic transition from massive (zone I) to finely laminated (zone II) deposits by a decade, probably not because the diatom flora responded slowly to 1890s lake-level rise and establishment of meromixis, but because previously buried shallow-water diatoms were resuspended during the transgression and redeposited offshore. Modified from Verschuren et al. (1999a).

et al. (2000a) suggested that this reflects the threshold on salt removal determined by the elevation of the sill separating Lake Oloidien from the main basin of Lake Naivasha. It is also possible, however, that the 1960s transgression caused some reworking of 1950s lowstand deposits containing halophilic diatoms.

In nearby Lake Sonachi, the diatom community responded quickly to a major 1890s lake-level rise leading to stable density stratification of the water column, but in the sediment record this response appears delayed by almost a decade because of redeposition during the transgression of previously buried shallow-water diatoms (Fig. 5; Verschuren et al., 1999a). Further, in all three groups of aquatic invertebrates studied (ostracods, cladocerans and chironomids), only a limited number of species appeared to respond strongly to salinity change itself; or actually, the osmotic stress associated with it. Most species responded more strongly to the substrate changes associated with lake-level change, or to changes in the distribution of aquatic vegetation, which itself is a function of both lake level and salinity (Verschuren et al., 2000b). While it is well known that lake depth, salinity, and substrate quality are all important factors in structuring aquatic invertebrate communities, it is sobering to realise that at the time scale of climate events sought for in modern high-resolution paleoclimate studies, the intuitive co-variation among these factors may be strongly modulated by transient system dynamics. Nonetheless, aquatic biota remain powerful proxy indicators of past hydrological change, especially in combination with one or more non-biological climate proxies.

(Semi-) quantitative lake-level inference

Provided that the calibration data set is developed from a suitably diverse collection of reference lakes, fossil diatom species composition can also be exploited for quantitative reconstruction of former lake-level changes (Cumming et al., 2002), succeeding a long tradition of more qualitative diatom-based approaches for lake-level reconstruction (see Wolin & Duthie, 1999). However, Birks (1998) suggests that because the relationship between diatom species composition and lake level much depends on local lake morphometry that may not be adequately represented in the reference data set, lake-depth inference models may best be calibrated with a collection of surface-sediment samples taken along depth transects in the study lake itself. However, this approach increases the risk of no-analogue fossil assemblages down-core (Cumming et al., submitted), particularly when lake-level fluctuations are accompanied by water-chemistry change. Cumming et al. (submitted) recommend both the use of calibration data sets in which lakes represent the full modern-day environmental gradients, as well as knowledge on how diatom floras change with depth within the lake in question. As with salinity-based climate inference, the relationship between lake-level and hydrologic balance can be exceedingly complex, requiring site-specific modeling efforts to transform lake-level data into paleoclimate records relevant to the paleoclimate community.

Quantitative diatom-based lake-depth reconstruction has so far not been attempted in Africa. Trials (K.R. Laird et al., unpublished data) suggest that diatom-based lake depth and/or surface-area inference is feasible for the chemically uniform subset of East African lakes but will require expansion of the current data set (Gasse et al., 1995) with more linked diatom and lake-morphometric data. Chironomid-based depth-inference models for African lakes are also now being developed (H. Eggermont & D. Verschuren, unpublished data). In the case of diatoms, lake depth and surface-area inference is based on the relative abundances of near-shore (typically epiphytic or benthic) and pelagic (open-water) species, their habitat preferences being determined by local differences in nutrient availability, water-column turbulence and transparency, or substrate texture. In the case of chironomids, depth inference is based on the relative abundances of littoral and shallow- and deep-water offshore species, and how the importance and composition of the offshore component is influenced by seasonal or permanent oxygen loss in the lower water column. One expected complication is that in permanently stratified lakes, persistent anoxia of the water column below the thermocline eliminates the deep-water faunal component, such that fossil assemblages deposited at deep-water coring sites will consist exclusively of shallow-water species, and may thus corrupt the reconstruction of lake-depth variation through time (Hofmann, 1998). A comparable problem may complicate diatom-based lake-depth reconstruction in relatively deep but turbid lakes. At short time scales, erosion and redeposition of shallow-water muds with their associated fossil flora and fauna during regressive-transgressive cycles (cf. section 5) may also here cause additional complications.

Given that lake-level fluctuations impact the species composition of lake biota mostly through associated changes in water-column dynamics and the distribution of various types of substrate (besides the osmotic stress caused by water-chemistry changes), the question arises why climate-driven changes in a lake's physical limnology cannot be reconstructed more directly from the composition and texture of the sediments in which the biological fossils are buried.

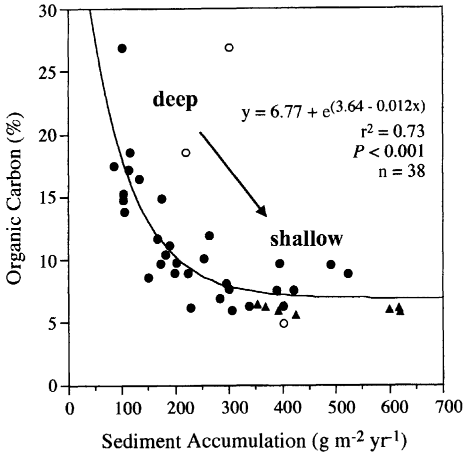

Figure 7. Relationship between sedimentary organic-carbon content and ^{210}Pb-derived sediment accumulation rate over the past 150 years (~AD 1840–1993) in a mid-lake core from Lake Sonachi (Kenya). The transition from decreasing to near-constant organic-carbon content (~8%) occurs when lake depth is ~4 m, the modern chemocline depth. Triangles represent low-organic muds deposited during an 1870–1880s lowstand when water depth was less than 2 m and random sediment redistribution occurred. Open circles are passive data points representing event horizons. Modified from Verschuren (1999b).

Climate reconstruction based on lithostratigraphical analysis of lake deposits has a long tradition (Lundqvist, 1927; Richardson, 1969; Digerfeldt, 1986), but high-resolution interpretations have often been qualitative and ambiguous, hampered by incomplete understanding of the local hydrological and sedimentation dynamics that control such basic sediment features as organic and inorganic carbon content. Yet relatively simple analyses of the local relationships between basin morphometry, physical limnology, and local sedimentation regimes, supplemented by short-core studies of how these relationships have changed through time due to historical lake-level fluctuation, can add considerable rigour to the interpretation of sediment composition, and permit (semi-) quantitative reconstruction of climate-driven lake-level change at decade-to-century time scales.

One basic principle is that the organic-carbon proportion of offshore lake sediments is usually not a function of organic production (mostly in-lake algal productivity), but reflects the dilution of organic matter by mineral sediment input, and/or exposure to oxidation before its permanent burial (Rowan et al., 1992). Progressive lake-level decline in a small strati-

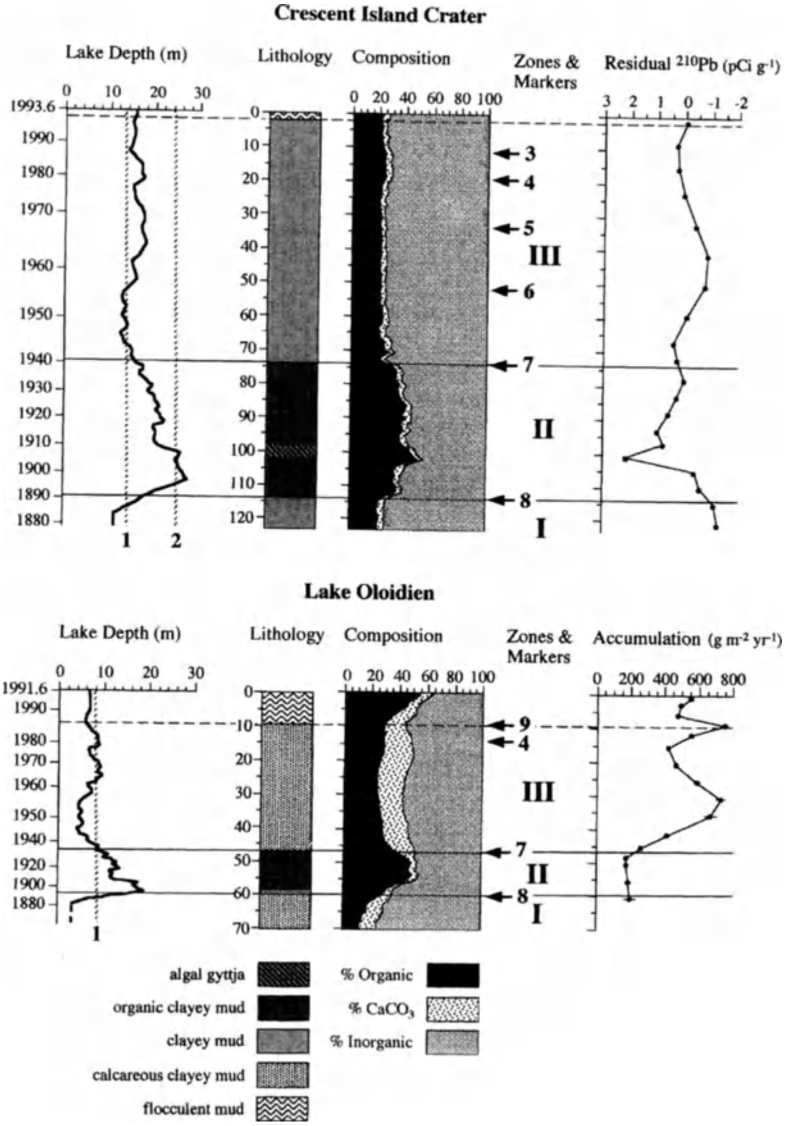

Figure 6. Relationship between lake depth, sediment composition, and sedimentation rate over the past 120 years (~AD 1870–1993) in mid-lake cores from Lake Oloidien and Crescent Island Crater in Lake Naivasha (Kenya). Organic-matter content is positively correlated with lake depth in a sequence from low-organic clayey mud to algal gyttja. Numbered arrows show historical marker horizons. From Verschuren (2001).

fied lake first causes increased focusing of low-organic (because partly oxidised) shallow-water sediments to offshore profundal areas, which dilutes the organic matter derived from pelagic algal production that has settled locally. In this phase, organic-matter content is inversely related to sediment-accumulation rate (Verschuren, 1999b; Fig. 6). By the time the lake has become so shallow that its entire bottom is well-aerated and subject to wind-driven turbulence, accelerated oxidation of sedimentary organic matter coupled with frequent horizontal redistribution has reduced organic-matter content to a constant level, representing the more refractive organic materials which remain. As a result, organic-carbon content is positively correlated with lake depth at the time of deposition in a lithological sequence from inorganic clayey mud to highly organic algal gyttja (Verschuren, 2001; Fig. 7). In a typically productive tropical lake, oxygen in warm (>20 °C) waters below the thermocline is consumed quickly, so that thermocline depth places a pronounced threshold on the depth gradient of organic-matter oxidation. Since thermocline depth among a set of lakes located within a single climatic region is primarily controlled by effective wind fetch (e.g., Shuter et al., 1983) and the wind shelter provided by surrounding topography (Melack, 1978), thermocline depth can be calculated, and transitions between sediment types in profundal areas can be tied to specific total depths of the water column. With certain limitations, this permits quantification of the absolute magnitude of past lake-level changes, at decadal to century-scale resolution (Verschuren, 2001). At the short end of this time scale, reliability of lake-depth inference based on percent organic matter alone will again suffer from hysteretic effects in sedimentation dynamics during a regressive-transgressive cycle; caution is also recommended when extrapolating a historical relationship between sediment composition and lake level to periods beyond the current lacustrine episode (i.e., prior to a desiccation phase), as this may have affected basin morphometry (Verschuren, 2001). Interpretative support from grain-size data and authigenic minerals can help to constrain the limits of this methodology. On the other hand, trustworthy lake-level inferences, even of a semi-quantitative nature, have the advantage over salinity inferences to more directly reflect the climate-driven changes in moisture balance that are the subject of climate reconstructions.

Conclusion

Climate reconstruction is arguably the most difficult discipline in paleolimnology. In no other application of paleolimnological methods is the cascading chain of cause and effect linking a sedimentary proxy indicator to the variable of interest so long and complex. No other application needs to strain the limits of chronological methods so much to produce a valid story. And most other applications typically do not deal with a long sequence of events, but can be content to identify pre-disturbance, disturbance, and recovery phases. Partly because of the complexities involved, some commentators on the future of paleoclimatology (e.g., Broecker, 1997; Berger & Maslin, 1999) do not envision a prominent role for lake-based climate reconstruction. But given the low potential of tree-ring and ice-core archives in tropical regions and the restricted distribution of high-quality cave speleothems, achieving the world-wide coverage of regional climate histories that is crucial to understand decade- to century-scale climate variability will not be possible without optimal exploitation of tropical lake records.

Lake-based paleoclimatologists must keep focus on the principal objective of high-resolution paleoclimate research, which is to produce histories of local climate variability of such quality that climate modellers can use them to constrain and validate the models being developed for long-term climate prediction. Strong, trustworthy paleodata yield unique insights in the patterns and modes of climate variability that can not be gained from instrumental meteorological data alone, and can compel modellers to produce a mechanistic explanation for them. With this in mind, all the methodological difficulties addressed in this paper reduce to two major challenges. The first is to produce high-resolution records of climate-proxy indicators whose particular relationship with climate variability is sufficiently well understood for them to validly represent climate history. Lake histories with high stratigraphical resolution cannot be treated as high-resolution climate records if the link between the climate-proxy signals and climate variability at the relevant time scale is not properly understood. The second is to produce local climate reconstructions that are fully independent with respect to both chronology and proxy-record validation. Climate-proxy records cannot claim their place in a regional or continental network of reconstructions when their interpretation or chronology is a priori tuned to or dependent on other records, either within or outside that region.

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The life-cycle of the asexual ostracod *Darwinula stevensoni* (Brady & Robertson, 1870) (Crustacea, Ostracoda) in a temporate pond

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Abstract

The life-cycle of the ancient asexual ostracod *Darwinula stevensoni* was studied during 1 year in a eutrophic pond in Belgium. The reproductive period of this species started in March and was effectively completed by September of the same year. All changes in population structure took place during the spring and summer months and a rapid turnover of the instars was observed. The life-cycle of *Darwinula stevensoni* appears to take one year or less in Belgium and this is considerably shorter than the 4 years which had been reported previously from subarctic populations. The difference to the present study is most likely temperature-related. Maximal densities of *D. stevensoni* were observed in June and July and attained 10^5 ind. m^{-2}. During winter, densities were lower with a mean of 10^4 ind. m^{-2}. Consequently, the calculated population size of each month was high throughout the year. Together with the low mutation rate, such a large population size could effectively counteract the stochastic loss of mutation-free genotypes as predicted by Muller's ratchet. *D. stevensoni* is a brooder; the maximum number of embryos and juvenile instars (up to third stage) found within a single female was 11.

Introduction

Within the extant non-marine ostracod lineages, different reproductive strategies exist. For example, some ostracod groups have sexual and parthenogenetic populations in different geographical areas a phenomena known as geographical parthenogenesis (Horne et al., 1998a). Species in other lineages are either exclusively sexual or asexual. The family Darwinulidae is a long-term asexual ostracod lineage and has received renewed scientific interest during the past decade. Darwinulids are brooders and all but one species have an externally visible brood pouch in the female (Rossetti & Martens, 1998): this leads to a distinctive carapace shape, which can be used to infer the gender of fossil specimens. From fossil data, no evidence of the presence of males in darwinulids has been found for at least 200 million years (Martens et al., 2003). Darwinulid ostracods thus constitute one of the few examples of animal groups that seem to persist over long evolutionary timeframes without sex. Together with the bdelloid rotifers and oribatid mites (Mark Welch & Meselson, 2000; Maraun et al., 2003) they defy current evolutionary theory, which predicts that fully asexual lineages are short-lived and evolutionary dead ends. They therefore were called ancient asexual scandals (Maynard Smith, 1978).

Darwinula stevensoni is the most common species of the family Darwinulidae and is found in deposits as early as the Miocene, about 25 million years ago (Straub, 1952). Because it is the oldest documented living ancient asexual species, it provides a genuine opportunity to investigate a number of important biological aspects associated with ancient asexuality. During the past decade, several relevant issues with regard to the biology of *D. stevensoni* have been studied (reviewed in Griffiths & Butlin, 1994; Martens, 1998) and the family Darwinulidae has been revised taxonomically (Rossetti & Martens, 1998). Some recent ecological studies have indicated that *D. stevensoni* shows all the characteristics of a general purpose genotype (GPG) (Rossi et al., 2002; Van Doninck et al., 2002). A GPG is a genotype capable of producing a broadly tolerant phenotype or a plastic response allowing the lineage to survive in a wide range of environmental conditions. The presence of a GPG may explain the observed wide geographical and ecological distribution of this ancient asexual and its persistence over long evolutionary timeframes (Van Doninck et al., 2002, 2003).

The population biology of *D. stevensoni* has thus far been studied in two oligotrophic, subarctic lakes: Gull Lake, situated in Lower Michigan Peninsula (42° N, 85° W) (McGregor, 1969) and Lake Pääjärvi, in southern Finland (61° N, 25° E) (Ranta, 1979). In Gull Lake, the life-cycle of *D. stevensoni* is reported to take more than 2 years with half of this time spent in the adult stage (McGregor, 1969). In Lake Pääjärvi, the life-cycle duration was 3–4 years (Ranta, 1979). These results indicate that *D. stevensoni* has an exceptional long life-cycle for such a small animal (length $<$ 1 mm) considerably longer than that of most other ostracods (Horne, 1983). In addition, this species is not rare (it is cosmopolitan and ubiquitous) and population densities are known to attain 10^5 individuals m^{-2} (Ranta, 1979). Both of the above studies on the population biology of *D. stevensoni* have been conducted in subarctic lakes which are characterised by long, cold winters and with ambient lake temperatures below 4 °C for most of the year. No similar studies have been conducted for other climatic regions.

Such studies are nevertheless urgently required, because exceptionally low substitution rates of nuclear regions and genes have been observed both within and between individuals and populations (Schön et al., 1998; Schön & Martens, 2003). This contradicts theoretical predictions on the genetic consequences of long-term asexuality as asexuals are supposed to be less efficient in purging their genomes from deleterious mutations (Kondrashov, 1993). The relevance of this mutation accumulation hypothesis depends strongly on the overall mutation rate of the organism. The latter is known to be affected by the number of cell divisions (Drake, 1996) and the metabolic rate (Martin & Palumbi, 1993), which are both influenced by the speed of individual development and life span. Indeed, an increasing genetic divergence of the nuclear *heatshock protein 82* from individual *Darwinula stevensoni* along a north-south gradient (with higher genetic divergence in the north) has hypothetically been attributed to longer life cycles in northern latitudes (Schön & Martens, 2003). The need to test this urges for life cycle studies of *D. stevensoni* at different latitudes throughrout Europe.

The stochastic loss of mutation-free genotypes in finite asexual populations, the so-called Muller's ratchet (Muller, 1964) is a second hypothesis predicting the accumulation of mutations in long-term asexuals. It can be countered by a low mutation rate and a high, effective population size. Accurate estimates of natural population densities of *Darwinula stevensoni* are therefore also required to test the importance of Muller's ratchet.

Here, we study the life-cycle and population dynamics of *Darwinula stevensoni* in a Belgian freshwater pond, i.e. in temperate conditions. The investigation of the life-cycle and population density of *D. stevensoni* in other than subarctic habitats is crucial for the elucidation of the most prominent genetic hypotheses on the prevalence of sex, namely mutation load and Muller's ratchet.

Materials and methods

Study area

The Grand Mellaerts (GM) pond is located in the village of Woluwé Saint Pierre, which is part of the agglomeration of Brussels, in the centre of Belgium (N 50° 49′ E 4° 26′). The following description of the pond is summarised from the report by Houvenaghel (2001). The GM has an area of 37×10^3 m^2 and a maximum depth of 2.5–3 m. It may be classified as eutrophic on the basis of its physical and chemical properties (total phosphorus = ca. 30 $\mu g\ l^{-1}$). The dissolved oxygen concentration ranges between 5.8 and 6.8 mg O_2 l^{-1}. The conductivity at 20 °C fluctuates between 630 and 780 $\mu S\ cm^{-1}$ and the pH between 7.6 and 7.7. Over the whole depth, the water is transparent throughout most of the year and there is a rich diversity of macro-invertebrates.

Sampling and sample processing

A 9 cm^2 internal tube, mounted on a gravity corer (type Kayak), was used for sampling. The uppermost 3 cm of sediments in each core were transferred to a plastic bottle (0.5 l). On each sampling date, 12 replicate cores or sampling units were taken, which were distributed over the pond according to a stratified random sampling program, resulting in 12 samples and 144 core units. The cores were labelled 2k, 4k, 6k, 8k, 10k, 12k, 14k, 16k, 18k, 20k, 22k and 24k (Fig. 1). All 12 cores were analysed separately.

In the laboratory, a solution of 70% ethanol (neutralised with borax till saturation) was added to each core. The sediment was subsequently fractioned through 3.15 mm and 0.125 mm sieves. Ostracods were retained on the 0.125 mm sieve. From each core (or sampling unit) all individuals of *D. stevensoni* were sorted by hand and counted under a binocular microscope. For each month sampled, the length of at least 85 free-living individuals was measured. These individuals were proportionally distributed over the twelve cores, i.e. more ostracods were measured from a core which contained a higher number of sampled ostracods in that month. Length measurements of *D. stevensoni* individuals were made using a Leica WILD M10 stereomicroscope with ocular micrometer. Each individual was placed laterally and the length of the right valve was measured. Embryos and juveniles within the female brood pouch were counted and measured after a dissection of the adult. No difference between the embryo stage and the first instars was made, because size alone did not allow distinction between these stages.

The sampling period spanned from 18 April 2001 to 30 April 2002. Sampling occurred monthly except for December 2001 when the pond was frozen over its entire surface. In total, 12 samples and 144 sampling units (or cores) were taken over a period of thirteen months. As depth in the GM pond is nearly constant over the whole habitat, no variation of abundance in relation to depth was studied.

Measurements and calculations

Water temperature was recorded continuously with an Escort Junior probe (Tech Innovators Ltd). This probe was placed at 1.5 m depth near the outlet of the pond. Temperature measurements of the water were conducted between the 8th of May 2001 and the 6th of May 2002. Mean weekly and monthly temperatures were calculated from these measurements, and minimum and maximum values were also determined.

For each month, the mean density and variance of the twelve cores was calculated (no. individual m^{-2}). The ratio of the variance to the mean gives a measure of the spatial distribution of the individuals (Elliott, 1977) in the GM population. A logarithmic transformation was applied to the mean monthly densities of the GM population prior to the calculation of 95% confidence limits. This is a prerequisite when sample size is small ($n < 30$) and the distribution contagious (see results; Elliott, 1977). The density values of each sampling unit were thus log (x+1) transformed; the mean, variance and 95% confidence limits of the transformed densities were then calculated with STATISTICA®, version 5. Finally, the factor derived from the logarithmic transformation was applied to the arithmetic mean of the samples to calculate 95% confidence limits. Arithmetic mean population sizes and area of the pond were used to calculate the monthly population density of *D. stevensoni* in GM. Minimum and maximum values are given. Size classes were determined from length measurements. Monthly, the proportion of each size class (over the twelve cores) was estimated and used to determine the density in each class based on the monthly mean arithmetic density (see above).

Results

Temperature

From May to August 2001, the mean water temperature in GM varied around 20 °C, with a maximum of 23.9 °C in early August (Fig. 1). The temperature then began to decrease and during winter (November till February 2002) water temperature fluctuated around a mean of 6.5 °C. By March–April 2002, the temperature again exceeded 10 °C (Fig. 1); at that time the reproductive period started. The lowest measured water temperature from GM during the sampling period, 1.7 °C, was recorded at the beginning of December 2001 (Fig. 1), when the lake was frozen over its entire surface.

Spatial distribution

That *D. stevensoni* showed a patchy distribution in GM was already obvious during the sampling, prior to further analysis, as large variation in abundance of this ostracod over the 12 areas of the sampling units was found. In Figure 2, the patchy distribution of April 2001 is presented as an example: some stations had no individuals (6k, 12k and 16k) whereas the highest density was observed in 22k (160 563 in-

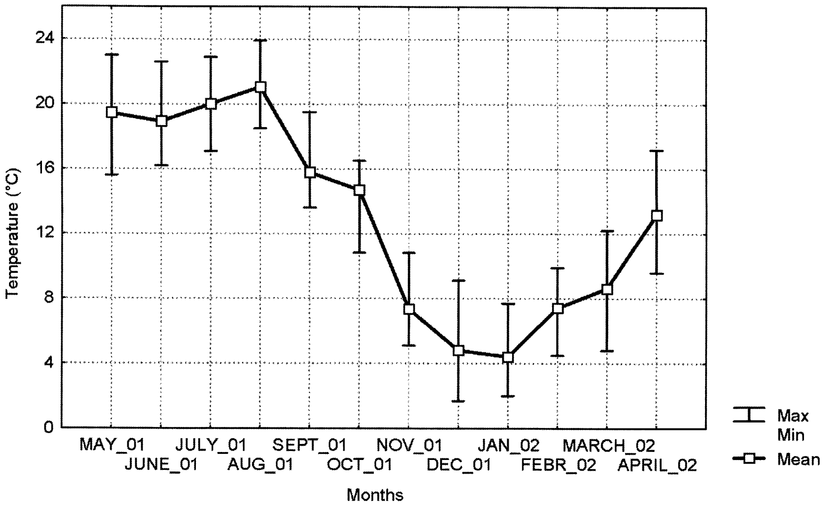

Figure 1. Mean, minimum and maximum water temperature (°C) of Grand Mellaerts pond, Belgium, for each month from May 2001 to April 2002.

dividuals m^{-2}). Thus, the spatial distribution within GM pond was contagious, with the variance to mean ratio always higher than unity. The maximum ratio was observed in June 2001 (1 308 585), the minimum in January 2002 (5897).

Densities and population size

Monthly population density estimates are shown in Figure 3. The logarithmic transformation was used to calculate the 95% confidence limits of mean densities. Confidence limits are not given in Figure 3, but instead are listed in Table 1.

The population densities showed a seasonal cycle. During the early summer months the numbers of *D. stevensoni* individuals increased. Most juveniles were observed within the brood pouch during June and July 2001 (Fig. 5). As a consequence, at that time also the highest mean densities were observed with 206 961 ind. m^{-2} in June and 172 742 ind. m^{-2} in July, respectively. The maximum density found in a single sampling unit of GM was 1 680 801 ind. m^{-2} (24k, 27.06.01). By August, the number of *D. stevensoni* individuals started dropping and, during winter, the population densities were considerably smaller than in summer and remained more or less constant (Fig. 3).

The estimated population size of *D. stevensoni* in GM was highest in June and July 2001. The maximal calculated population size was 7.66×10^{9} individuals

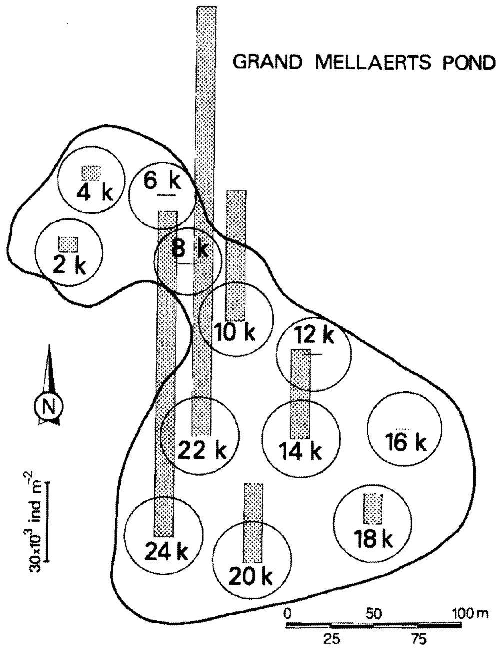

Figure 2. Position of the 12 sampling units (or cores) within the Grand Mellaerts pond. Bars at each station indicate the density of *Darwinula stevensoni* in April 2001.

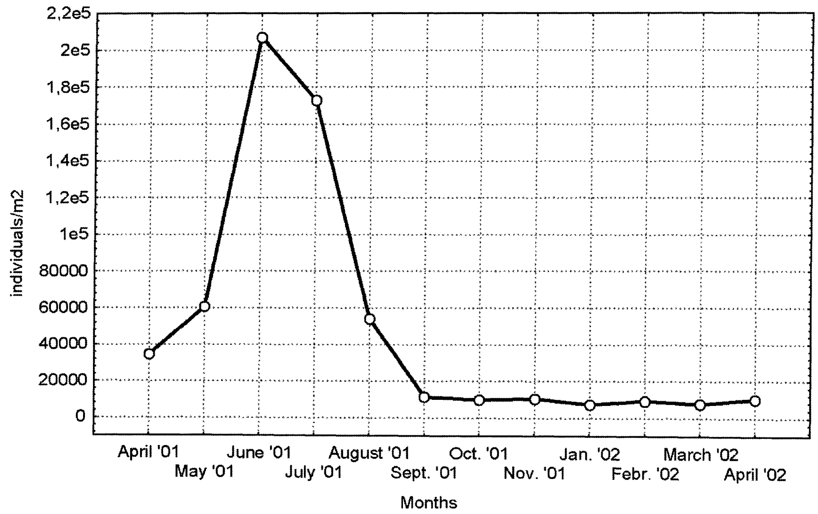

Figure 3. Mean abundance of the *Darwinula stevensoni* population (no. of ind. m^{-2}) during the study period. The 95% confidence limits are given in Table 1.

Table 1. The arithmetic mean density and 95% confidence limits (after logarithmic transformation) of the *Darwinula stevensoni* population in Grand Mellaerts pond for each sampling month

Month	Mean (10^3 inds m^{-2})	−95% (10^3 inds m^{-2})	+95% (10^3 inds m^{-2})
April '01	34.5	1.8	674.3
May '01	60.5	2.1	1708.7
June '01	207.0	6.1	7065.0
July '01	172.7	6.6	4546.9
August '01	53.9	1.7	1713.9
September '01	11.3	0.5	283.6
October '01	9.7	0.7	135.2
November '01	10.5	0.5	206.1
January '02	7.3	0.8	68.6
February '02	9.4	1.7	51.7
March '02	7.6	1.4	41.3
April '02	10.2	0.5	221.3

in June 2001, the observed minimum was 2.72×10^8 in January 2002.

Life cycle

In total, nine length classes were distinguished by their size (Table 2), the last class being the adult stage. These nine classes correspond to the nine instars known in most podocopid ostracods. The two smallest instars were found only within the brood pouch of the adult. The moult from the first to the second as well as from the second to the third instar takes place within the carapace of the mother, after which the third instar leaves the brood pouch. Here, the embryo stage is included in the first instar class. The size range of each instar stage and the calculated ratios between the lengths of one instar stage to the next larger one were in accordance with the results of Ranta (1979) (Table 2) although all Belgian instars were smaller.

The frequency distributions of the various instar stages of *D. stevensoni* over a period of 1 year are shown in Figure 4, whereas the amount of adult females with and without instars in the brooding pouch over the study period is illustrated in Figure 5. Juveniles of instar stage 1 were first observed inside the brooding pouch in March 2002 and their number increased in April. In April 2001, half of the sampled adult females were gravid (Fig. 5). In May 2001, the highest percentage of first instars was found. During this month, 76% of the measured adults were gravid with a mean number of 5.2 juveniles per mother (95% confidence limit: 4.3–6.0). By June 2001, most adults were gravid and first, second and third instars were found within the brood pouch (Fig. 5). Furthermore, 80% of the classified third instar juveniles were already free-living and some fourth instars were also

Table 2. Length (in mm) of the different classes (or instars) of the ostracod *Darwinula stevensoni*. For each instar stage, the length range (min.-max.) and the calculated mode are given; ratio = ratio between instar (i + 1) and i. The results of Ranta (1979) are given as comparison

Instar	GM (present study)				Ranta (1979)	
	Min.	Size	Max.	Ratio	Size	Ratio
1st	0.117	0.130	0.143	1.18	0.144	1.33
2nd	0.156	0.169	0.169	1.38	0.192	1.22
3rd	0.221	0.221	0.234	1.22	0.234	1.25
4th	0.260	0.273	0.286	1.14	0.282	1.15
5th	0.299	0.312	0.325	1.20	0.324	1.20
6th	0.351	0.364	0.390	1.23	0.390	1.22
7th	0.429	0.442	0.481	1.24	0.474	1.23
8th	0.532	0.545	0.597	1.30	0.582	1.28
9th	0.688	0.727	0.779		0.744	

detected (Fig. 4). In July 2001, adults with all three instar stages within the brood pouch were still found (Fig. 5), in August, only first and second instars. In addition, all size classes were present in the studied population in both July and August (Fig. 4), although frequencies differed between these two months. In August 2001, however, the proportion of gravid females had dropped considerably as compared to July, except for the number of females with only first instars (Fig. 5). At the end of September, no adults were gravid any more and the first three instar stages were absent from the population. Individuals now belonged to instar stages four till nine, with instar fourth being the least abundant (Fig. 4). This population structure remained almost unchanged during the autumn and winter months. Only in March 2002 appeared the first instars again in the brood pouch. By April 2002 these first instars had increased in frequency and most of the observed adults were again gravid (Figs 4 and 5).

Discussion

Life-cycle

The two cohorts observed in April 2001 and 2002, i.e. a cohort of first instar juveniles and a separate cohort with juveniles belonging to the classes four till nine indicate that the life-cycle of *Darwinula stevensoni* takes less than 1 year. This is also supported by the presence of all instars in the studied population by July 2001. The transition from one instar to the next during the 5 months of the reproductive period was rapid and continuous: the first instars from March and April already attained adult stage by August–September. In August, almost no third instars were present anymore while first and second instar juveniles still abounded; this discontinuity indicates that a few of the instars which developed into adults during spring already reproduce in the same year. The constant low abundance of the third instar stage compared to the second and fourth in June and July may be explained by different developmental times of these instars. The low abundance is most likely not caused by an increased mortality through the transition to a free-living stage. Higher numbers within the brood pouch than free-living would be expected, whereas the opposite, namely exclusive free-living of third instars, is observed in August 2001 (compare Figs 4 and 5).

Thus, reproduction of *D. stevensoni* in GM takes place more than once in a year. During winter, no change in the population structure of *D. stevensoni* in GM was observed and the development of the animals was arrested. After winter (March), the maturation of each instar continued; furthermore, first instars appeared within the brood pouch of the adults.

The maximum number of juveniles observed per single adult in GM pond was 11. This is the same as in Lake Pääjärvi (11; Ranta, 1979) but less than in in Gull Lake (15; McGregor, 1969). These numbers do not necessarily reflect the total number of offspring per adult, because there is a dynamic balance between the release of juveniles out of the brood pouch and the production of new ones. Gandolfi et al. (2001) demonstrated, in laboratory controlled conditions, that *D. stevensoni* has a maximum of 12 juveniles per clutch per female and 0.02–0.07 eggs were layed per day. This is lower than in other freshwater ostracods: the egg laying rate of *Heterocypris incongruens*, for example, is between 2.5 and 6 per day, depending on the clonal lineages, and about 1.2–1.5 for *Eucypris virens*. In cultures of *H. incongruens* and *E. virens*, a maximum of 60 and 71 eggs, respectively, per female has been observed (Van Doninck, unpubl. data). The ratio of egg size to adult body size is about 0.2 in *D. stevensoni*, while it is c. 0.1 in *H. incongruens* and *E. virens* (Van Doninck, unpubl. data). The life-cycle of *D. stevensoni* appears to be longer than that of other freshwater ostracod species. Furthermore, it has been well-documented that active brood care occurs in *D. stevensoni* (Horne et al., 1998b). These fecundity data seem to indicate that *D. stevensoni* fits into the K-selected end of the r-K continuum.

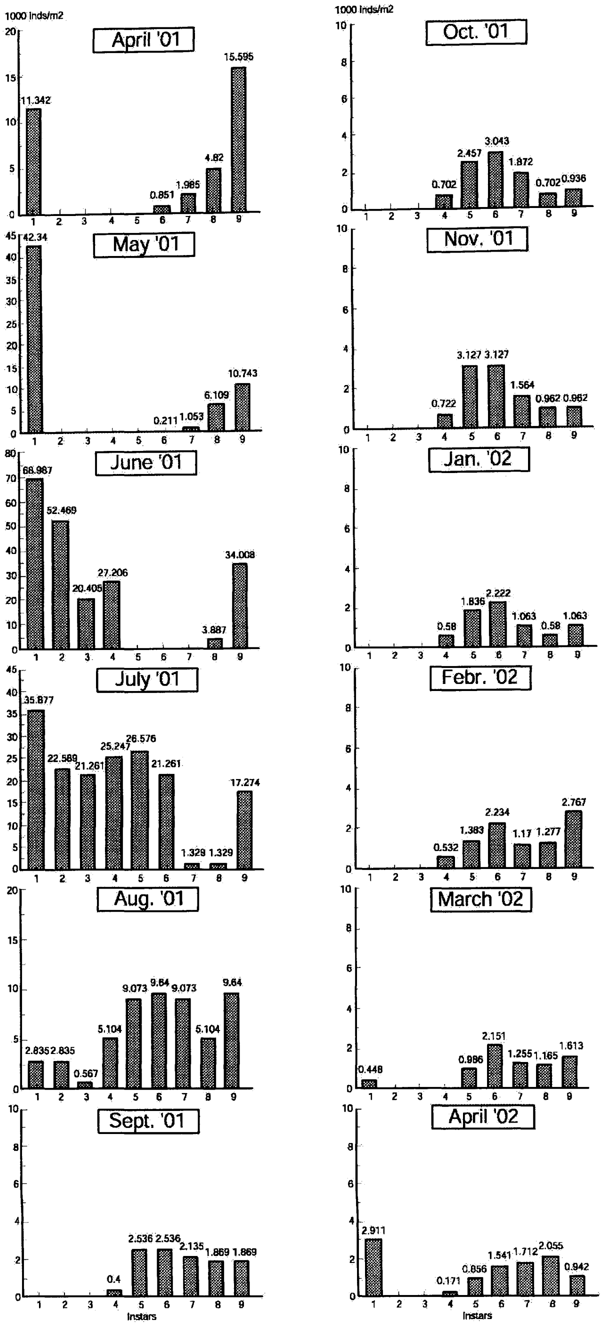

Figure 4. Size-frequency histograms of the *Darwinula stevensoni* population in Grand Mellaerts pond (April 2001–April 2002): proportions of mean densities in function of the different instars. Note the different vertical scales.

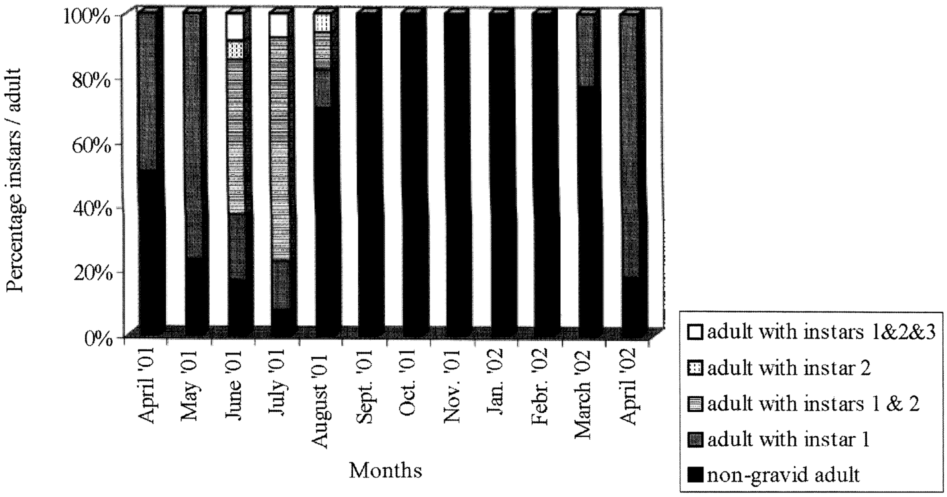

Figure 5. Percentages of the 9 different instars within the brood pouch of the adults (A).

Factors influencing the life-cycle duration

The observed cessation of maturation and reproduction during winter may be temperature-related. When the reproduction ceased in September 2001, however, the water temperature was still ranging between 13.6 °C and 19.5 °C, whereas in March 2002 adults began to have first instars at temperatures from 4.8 °C onwards. Therefore, we expect that other factors may also regulate the reproduction of *D. stevensoni* in GM. One of these may be photoperiodicity, which is, on a multi-annual basis, a constant factor at each latitude.

According to McGregor (1969) the reproductive potential of *D. stevensoni* in Gull Lake is correlated with temperature and varies with depth. At depths of 6 m, the annual reproductive period of *D. stevensoni* started in May and ended in October. McGregor (1969) found a complete turnover of adults each year and first and second reproductive season adults were present. However, the different juvenile classes were not distinguished and the population dynamics of the instars was not studied. As a consequence, the analysis of the life-cycle of *D. stevensoni* in Gull Lake remains incomplete. In the study of Ranta (1979) in Lake Pääjärvi, the first instars of *D. stevensoni* appeared within the brood pouch at the end of May–June, thus later in the year than in GM. By September of the first year, the juvenile cohort consisted of third and fourth instars, and remained unchanged during the first winter. At the end of the winter period (June), these individuals matured further till fifth and sixth instars, which then persisted over the second winter. At the end of the second summer, some of these individuals became adults. Maturation of *D. stevensoni* in Lake Pääjärvi occurred during the summer months (June–September) when temperatures fluctuated around 20 °C. In the study of Ranta (1979), five to six instar stages and at least three different cohorts were present at any time. Thus, the life-cycle of *D. stevensoni* in Lake Pääjärvi takes at least 3 years.

The life-cycle of *D. stevensoni* in GM and Lake Pääjärvi is clearly different. Such differences in life-cycles between populations are not necessarily genetically fixed but may result from selective effects of particular environmental parameters varying between the two habitats (Schwartz, 1984). Here, temperature seems an important factor: for most of the year the temperature in Lake Pääjärvi is below 4 °C with the lake being frozen from December to May (Ranta, 1979; Sarvala, 1979), whereas in GM it rarely attains such low temperatures. Thus, for *D. stevensoni* it appears that the life-cycle completed at warmer temperatures results in earlier maturation and earlier reproduction. This observation has also been reported for *Daphnia* (Schwartz, 1984). However, other factors such as photoperiodicity may also affect the life-cycle (see above). The influence of higher temperatures in the Belgian pond, resulting in faster growth of the *D. stevensoni* individuals, may cause a generally smaller size which may explain the size difference of individuals from GM compared to Lake Pääjärvi.

Population size

In the present study, the densities of *D. stevensoni* in GM were estimated monthly. High densities were observed in the months June and July 2001, attaining 10^5 ind. m^{-2}. During the winter months, densities were lower (10^3–10^4 ind. m^{-2}). In the study of Ranta (1979), the maximum density was observed in September and comprised 160 000 ind. m^{-2}, the minimum density was 30 000 ind. m^{-2} (June). These observations indicate high population densities of *D. stevensoni* in both GM and Lake Pääjärvi.

Bell (1982) stated that a population size of 10^{10} may be sufficient to avoid the effects of Muller's ratchet in the absence of sex. From the density estimates, the population size of GM was calculated and attained on average 10^9–10^8 individuals, which is close to the limit of 10^{10}. However, it is not known whether these large population sizes are maintained for extended periods of time.

Recently, Schön & Martens (2003) demonstrated from DNA sequence data that nuclear substitution rates of of *D. stevensoni* are exceptionally low, contradicting theoretical expectations from Muller's ratchet (Muller, 1964) and the mutational load (Kondrashov, 1993) hypotheses. The operation of the ratchet might be slowed down or even stopped by the observed, large population size. Although the life cycle of *D. stevensoni* in temperate conditions is not as long as in (sub) arctic environments, it is still much longer than in other freshwater ostracods. Also, considerably less eggs per female are produced in *D. stevensoni* than in other ostracods which might translate into a lower number of cell divisions. Both factors will further lower the mutation rate of *D. stevensoni.* In addition, there are indications, at least for *D. stevensoni,* that active brood selection is present (Horne et al., 1998b). Brood selection is a way to selectively abort defective offspring, which increases the mean fitness of the brood (Lively & Johnson, 1994) and could this way further reduce the mutational load in offspring. All of these different strategies, adopted by *D. stevensoni* may contribute to the long-term persistence of this species without sex.

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Studying effects of some surfactants and detergents on filter-feeding bivalves

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Abstract

Effects of several surfactants and chemical mixtures on marine bivalves were studied. An anionic surfactant, sodium dodecylsulphate (SDS), and a cationic surfactant, tetradecyltrimethylammonium bromide (TDTMA), inhibited the filtering activity of oysters (*Crassostrea gigas*). Similar effects were exhibited by some chemical mixtures that included surfactants. Those mixtures inhibited the filtering activity of *Crassostrea gigas* and *Mytilus galloprovincialis.* The new results are in agreement with the author's previous experiments, where a number of xenobiotics and/or pollutants inhibited the filtering activity of several species of marine and freshwater bivalves, e.g., it had been shown that SDS inhibited filtering activity of *Mytilus edulis* (e.g., Ostroumov, 2000c, 2001a). This experimental approach is helpful in assessment of environmental hazards from man-made chemicals that can contaminate marine systems.

Abbreviations: LD – liquid detergent; SDS – of sodium dodecyl sulfate; TDTMA – tetradecyltrimethylammonium bromide; SD – synthetic detergent

Introduction

It has previously been shown that some amphiphilic chemicals (surfactants) and surfactant-containing chemical mixtures affect filtering activity of mussels (e.g., Ostroumov et al., 1997, 1998). Taking into account the ecological importance of this effect (e.g., Ostroumov, 1998, 2000a,b,c), it was of interest to broaden the study of this issue and to determine whether some other organisms important for aquaculture may be affected by those compounds.

It should be underlined that bivalves are among the most important cultured marine species. The average annual production of marine bivalves grown in aquaculture significantly exceed that of marine fish (9.7-fold) and crustaceans (4-fold) grown in mariculture (World Resources..., 1994). Owing to the importance of mariculture, it is necessary to determine the factors that may disturb some physiological activities of objects grown in mariculture (including bivalves) and deteriorate their living and culturing conditions.

The aim of this study was to determine whether some surfactants and surfactant-containing chemical mixtures inhibit the filtering activity of the important bivalve species grown in mariculture, namely the oyster *Crassostrea gigas* Thunberg.

The experiments were performed according to the previously described procedure (Ostroumov et al.,1998; Ostroumov, 2001a, b) with some slight modifications. The filtration rate was determined by the decrease in the optical density of the incubation medium at 550 nm as a result of the removal (due to filtration) of *Saccharomyces cerevisiae* cells that were preliminarily added to the marine water. The concentration of *S. cerevisiae* cells (SAF-Moment, S.I. Lesaffre, 59703 Marcq-France) was 100 mg l^{-1} (dry weight). The temperature is indicated in the tables. The optical density was measured using the SF-26 LOMO spectrophotometer, the optical way length was 10 mm. The average weight of the oysters and the volume of the incubation medium are indicated in the notes to the tables. The oysters were grown at the mariculture farm of the Institute of South Sea Biology

Table 1. Inhibition of the *C. gigas* filtering activity and uptake of unicellular organisms from water by TDTMA (0.5 mg l^{-1})

Measurement, No.	Incubation time, min	Optical density at 550 nm			B/A, %
		Variant A (control, without TDTMA)	Variant B (with TDTMA)	Variant C (control, *S. cerevisiae* alone, without bivalves and TDTMA)	
1	5	0.080	0.194	0.307	242.5
2	11	0.043	0.148	0.305	344.2
3	20	0.018	0.137	0.303	761.1

Note: Each experimental beaker contained 10 one-year-old bivalves. Total wet weight of the bivalves with shells was 47.3 g and 55.2 g in beakers A and B, respectively. Incubation temperature was 27 °C. The volume of the incubation medium was 500 ml.

Table 2. Inhibition of the *Crassostrea gigas* filtering activity and uptake of unicellular organisms from water by SDS (0.5 mg l^{-1})

Measurement, No.	Incubation time, min	Optical density at 550 nm			B/A, %
		Variant A (control, without SDS)	Variant B (with SDS)	Variant C (control, *S. cerevisiae* alone, without bivalves without SDS)	
1	4	0.117	0.181	0.176	154.70
2	12	0.074	0.156	0.179	210.81
3	20	0.048	0.111	0.174	231.25
4	29	0.035	0.074	0.164	211.43

Note: Each experimental beaker contained 16 one-year-old bivalves. The total wet weight of the bivalves with shells was 23.5 g and 23.6 g in beakers A and B, respectively. Incubation temperature was 23 °C. The volume of the incubation medium was 250 ml.

Table 3. Some chemicals that have an adverse effect on the filtering activity of the bivalves

Measurement	Chemical (described in the text)	Organism	Reference
1	SDS	*C. gigas*	New data
2	TDTMA	*C. gigas*	New data
3	SD1 (L)	*M. galloprovincialis, C. gigas*	New data
4	LD1 (E)	*M. galloprovincialis, C. gigas*	New data
5	LD2 (F)	*M. galloprovincialis, C. gigas*	New data
6	SD2 (I)	*M. galloprovincialis*	New data
7	SDS	*M. edulis*	Ostroumov et al. (1997)
8	SDS	*M. galloprovincialis*	Ostroumov (2000c)
10	Triton X-100	*M. edulis*	Ostroumov et al. (1998)
11	Triton X-100	*Unio tumidus*	Ostroumov (2001a)
12	TDTMA	*U. pictorum*	Ostroumov (2001a)

(National Academy of Sciences of Ukraine) and the State Oceanarium of Ukraine.

We analysed some effects of sodium dodecyl sulfate (SDS), tetradecyltrimethylammonium bromide (TDTMA), several synthetic detergents (SDs), and liquid detergents (LDs) on bivalves. In this paper, the following denotations are used: SD1(L), Lanza-automat (Benckiser); SD2(I), IXI Bio-Plus (Cussons);

Table 4. Filtration of water by bivalves (some examples)

Organism and measurement unit	Values measured	Comments, references
Mytilus galloprovincialis, % of suspension removal from the 3-m near-bottom water layer during 6 h	20%	The measurement and estimates refer to the shelf ecosystem of the northern Black Sea, taking into attention the real size structure of mussel populations (Zaika, 1992)
Unioniidae, the volume filtered by the bivalves of 1 m^2 of the bottom, per day	0.14 m^3 m^{-2} day^{-1}	The Hudson River estuary (Strayer et al., 1999)
Dreisseniidae, the volume filtered by the bivalves of 1 m^2 of the bottom, per day	0.1–5 m^3 m^{-2} day^{-1}	Rivers and lakes of North America (Strayer et al., 1999), a range of various data
Dreisseniidae,% of the filtered volume of water column per day	70–125%	(Strayer et al., 1999), during the summer vegetation period

LD1 (E), dish washing liquid E (Cussons International, Ltd.); and LD2 (F), dish washing liquid Fairy (Procter & Gamble, Ltd.).

We discovered that a typical cationic surfactant containing a tertiary ammonium group, TDTMA, inhibited the filtering activity of *C. gigas* (Table 1). The optical density of the cell suspension differed from the control more than twofold 5 min after the addition of TDTMA. The optical density differed from the control more than sevenfold (by the factor 7.6) 20 min after the addition of the cationic surfactant.

SDS is an important anionic surfactant contained in numerous industrial mixtures that contaminate water bodies. We discovered that SDS also inhibits the filtering activity of *C. gigas:* after 12–29 min of incubation with SDS, the concentration of the cell suspension differed from the control more than twofold (Table 2).

Similar results were obtained when some surfactant-containing mixtures were tested (Table 3). To enable the comparison and to provide a more comprehensive vision of the problem, Table 3 includes the data obtained not only with the oysters, but also when studying some other bivalves important for mariculture, namely, the mussels *Mytilus edulis* and *Mytilus galloprovincialis.*

All experiments showed that the decrease in water turbidity due to the filtration by the bivalves was associated with the formation of pseudofecal pellets, which precipitate onto the bottom. When the filtration rate reduced as a result of the effect of the surfactant or surfactant-containing mixture, the amount of pellets decreased.

We would like to emphasise two points concerning the results of this study. First, the inhibitory effects demonstrated in this study were observed at the surfactant concentration of 0.5 mg l^{-1} or higher, which was actually detected in some polluted aquatic ecosystems (Review on... 1976).

Second, the rate of water filtration by invertebrates is relatively high (Alimov, 1981; Zaika, 1992; Dame, 1996; Strayer et al., 1999; and Table 4). The filtration of water has some conditioning effect on aquatic ecosystems (Alimov, 1981; Ostroumov, 2001a, 2002). The decrease in this effect and filtering activity has a significant impact on the state of ecosystems. The biomass of mussels in natural marine habitats in the Black Sea exceeds 1 kg m^{-2} (Zaika, 1992). The biomass of bivalves in some marine habitats was found to be up to over 10 kg m^{-2} (with shells) (Ostroumov, 2001a, 2002). In mariculture, the biomass of bivalves per unit area may be higher, and the effect of filtering activity and its alteration on the ecosystem can be even more pronounced. Parameters, characteristics, and components of aquatic ecosystems that may be significantly influenced by the filtering activity of the bivalves include microzooplankton (the number and composition), macrozooplankton, phytoplankton, bacterioplankton, suspended solids, dissolved inorganic nitrogen (DIN), transparency (Secchi discs), and soluble reactive phosphorus (SRP) (Dame, 1996; Ostroumov et al., 1997; Strayer et al., 1999; Ostroumov, 2000c).

The threat of a disturbance of the filtering activity should be taken into consideration when the evaluation is made of the ecological hazard of the chemical pollution of aquatic ecosystems (Yablokov, Ostroumov, 1985, 1991; Ostroumov, 1986, 2001).

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