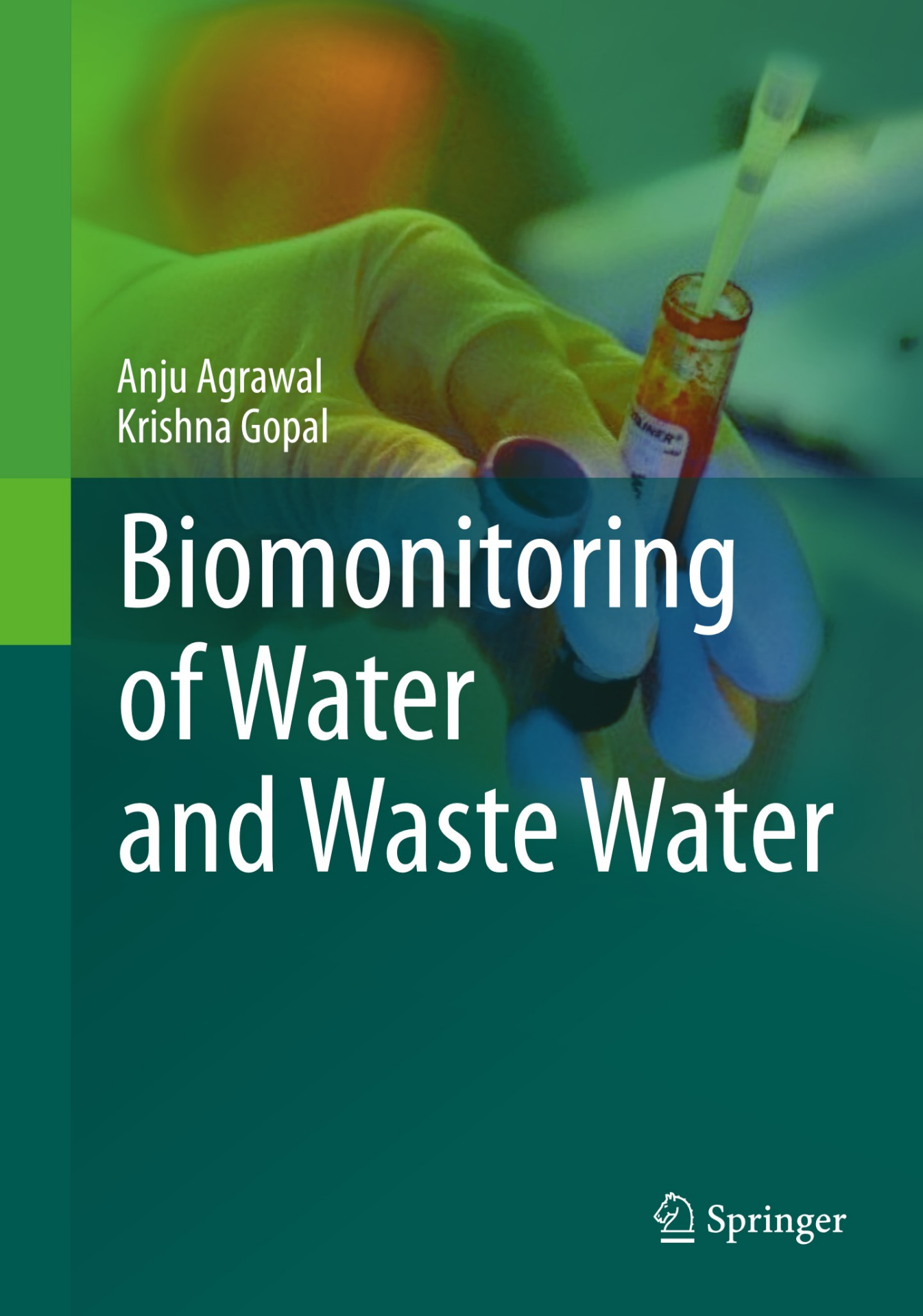

Anju Agrawal
Krishna Gopal

Biomonitoring of Water and Waste Water

Springer

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Anju Agrawal • Krishna Gopal

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Foreword

The present textbook entitled *Biological Monitoring of Water and Waste Water* is an appreciable attempt made by Dr. Anju Agrawal, Associate Professor, and Dr. Krishna Gopal, Senior Principal Scientist. In fact, books written by Indian authors on Biological Monitoring and Waste Water are scanty. The books written by foreign writers are either not easily understood by Indian students or not accessible in market. Therefore, in the present national context such an attempt by two professionals are commendable.

Biological monitoring of water quality could be useful for assessing the overall health of water bodies followed by safe supply of drinking water. The chemical nature of toxicants is highly dynamic in the environment with time and space, whereas biological systems can integrate all environmental viables over a large period of time in terms of effect, which can be easily measured and quantified. In view of the above, there is pressing need to determine the biological quality of natural resources as well as drinking water based on standard protocols and guidelines provided by regulatory agencies.

This book fulfils the pressing need today in monitoring the biological responses of the species and communities, which are sheltering under the stress of abiotic and biotic alterations taking place inside the ecosystem. Apart from that, parameters selected within the chapters also cater to the needs of policy makers and authorities assessing for granting environmental clearances. In my opinion, the effort made by Dr. Agrawal and Dr. Gopal is praiseworthy. This book shall find a niche in the libraries and information centres of Indian universities and research institutes as well. I am sure the readers will like reading this book.

Directorate of Coldwater Fisheries Research

Bhimtal
May, 2012

Dr. P. C. Mahanta
(P.C. MAHANTA)

Preface

Biological monitoring of water quality could be useful for assessing the overall health of water bodies and safe supply of drinking water. The chemical nature of toxicant is highly dynamic in environment with time and space, whereas biological system can integrate all environmental variables over a large period of time in terms of effect which can be easily measured and quantified. In view of the above, there is pressing need to determine the water quality of natural resources as well as drinking water based on the standard protocols and guidelines from regulatory agencies.

It is clear that the synthetic chemicals are essential for our society to maintain the health and well-being of the people. However, there has been a range of detrimental effects on human health and natural environment. In general, we need to improve our management of waste chemicals discharged into the air, water and soil environments. New techniques are needed to predict adverse effects before they occur and for the treatment of wastes. In addition, a range of social, political and economic factors will be needed to be taken into account in order to achieve success.

The improper disposal of wastes causes contamination of both soil and water, which could lead to adverse effects on human health. Migration of chemicals through ground and surface water increases contaminants in drinking water sources. While giving the categories of hazardous wastes, there regulatory quantities and hazard potential in different types such as industrial, domestic, agricultural, nuclear, hospital, biochemical and pharmaceutical wastes.

Every known substance has the potential to be toxic, while a potent toxin could be useful to human health. Several biotoxins can be used as medicines and also as pharmacological tools because of their specificities. Biotoxins are produced by wide range of organisms for their own defence and offence. They can severely affect or kill other living organisms. They can enter the body through ingestion of food and water or by air through aerosols. Marine biotoxins are important since they are most potent and many of them get biomagnified and become part of food chain.

Heavy metals and other chemicals commonly found above the prescribed permissible limits and microbial agents have been found in drinking water. Disinfection is the most important step in the treatment of water for public supply for destruction of pathogens. It involves the use of a reactive chemical agent such as chlorine, which is not only bactericidal but in excess amounts is also capable of reacting with other water constituents to form new

compounds with harmful long-term effects of human health. There is a need for the development of a natural disinfectant with no possible long-term harmful effects. Conclusively, it is clear that most of the xenobiotics and toxins can be determined using sophisticated instruments. However, mechanical devices and proper particles are only tools to record the biological monitoring of a natural ecosystem.

This book *Biological Monitoring of Water and Waste Water* is divided into fourteen chapters. The first chapter pertains to Measurement of Primary Productivity in Relation to Food Chain. The second chapter deals with Aquatic Weeds in Occurrence and Distribution. The third chapter deals with the Analysis of Phytoplankton and Zooplankton: Its Qualitative and Quantitative. The fourth chapter deals with Application of Diversity Index in Measurement of Species Diversity. Fifth chapter outlines Challenges of Fish Diversity in Polluted Water. The sixth chapter includes Biomass Production in Food Chain and Its Role in Trophic Levels. The seventh chapter includes Concept of Rare and Endangered Species and Its Impact as Biodiversity. The eighth chapter includes Protected Areas in Relation to Marine Parks and Sanctuaries. The ninth one includes Principles of Statistics and Reporting of Data. The tenth one deals with the General Principles of Toxicity and Its Application. The eleventh one includes Fate of Biotoxins in the Environment and Its Health Implications. Twelfth deals with Microbial Toxicity Studies. The thirteenth one is on Toxic Cyanobacteria in Water and Their Public Health Consequences. The fourteenth one is on Good Laboratory Practices in Biomonitoring.

Over and above, these chapters have been selected keeping in view of the syllabus of the Indian Universities and guidelines of institutions. There is pressing need today to monitor the biological responses of the species and communities which are sheltering under the stress of abiotic and biotic alterations undergoing inside the ecosystem. Parameters selected within the chapters are also catering the needs of policymakers and authorities assessing for granting environmental clearance. We authors will be happy to know the suggestions from the readers and other stake holders. The authors are sure that this book will be very useful to students and scientific community as a whole. Thanks are due to Springer for publishing this book.

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About the Editors

Dr. **Anju Agrawal** did her Doctor of Philosophy from Lucknow University, Lucknow, in the year 1986, but her work was carried in Central Drug Research Institute, Lucknow. Presently she is working as Associate Professor in S.N. Sen B.V.P.G. College, Kanpur which is associated to Chattrapati Sahuji Maharaj University, Kanpur. She has 13 books to her credit and ten book chapters. Her current area of research is parasitology and toxicology. She has published number of papers in both National and International Journals of repute and presented her work in Symposia both abroad and in India. She has received number of awards namely Fellow of Academy of Environmental Biology and Sneh Lata Gold Medal.

Dr. **Krishna Gopal,** Senior Principal Scientist, Aquatic Toxicology Division, CSIR-Indian Institute of Toxicology Research, Lucknow, is a distinguished Toxicologist. During his long research journey, he has developed expertise on standardization of modern techniques to assess the severity and mechanism of toxicity and physiological consequences, viz. neurobehavioral, neurotransmitters and series of hormone induced tissues and hematological changes in freshwater fish exposed to xenobiotics. His current area of research is related to assessing waterborne diseases at different developmental stages, environmental risk on direct exposure and indirectly through food chain, drinking water quality, biotoxins and emerging pollutants of global concern.

He has been credited with two patents and more than 120 research papers in journals of repute, and most of them were cited in reputed journals. He has edited five books and more than ten book chapters in his research domain.

Measurement of Primary Productivity in Relation to Food Chain

1

1 Introduction

Primary production is the production of organic compounds from atmospheric or aquatic carbon dioxide, mainly through the process of photosynthesis. Nearly all life on Earth is directly or indirectly reliant on primary production. The organisms which are responsible for primary production are known as primary producers or autotrophs and form the base of the food chain. In terrestrial ecoregions, these are mainly plants, while in aquatic ecoregions, algae are primarily responsible. Primary production is the production of chemical energy in organic compounds by living organisms. The main source of this energy is sunlight, but a minute fraction of primary production is driven by lithotrophic organisms using the chemical energy of inorganic molecules. The energy is used to synthesise complex organic molecules from simpler inorganic compounds regardless of source such as carbon dioxide (CO_2) and water (H_2O). The following two equations are simplified representations of photosynthesis and chemosynthesis:

$$CO_2 + H_2O + \text{light} \rightarrow CH_2O + O_2$$

$$CO_2 + O_2 + 4H_2S \rightarrow CH_2O + 4S + 3H_2O$$

In both cases, the end points are reduced to carbohydrate (CH_2O); the molecules mainly are glucose or other sugars. These relatively simple molecules may be then used to further synthesise more complicated molecules, including proteins, complex carbohydrates, lipids, and nucleic acids, or be respired to perform work. Consumption of primary producers by heterotrophic organisms, such as animals, then transfers these organic molecules (and the energy stored within them) up the food web, fuelling all of the Earth's living systems.

2 Gross Primary Productivity and Net Primary Production

Gross primary production (GPP) is the rate at which an ecosystem's producers capture and store a given amount of chemical energy as biomass in a given length of time. Some fraction of this fixed energy is used by primary producers for cellular respiration and maintenance of existing tissues. The remaining fixed energy is referred to as net primary production (NPP):

$$\text{NPP} = \text{GPP} - \text{respiration by plants}$$

Net primary production is the rate at which all the plants in an ecosystem produce net useful chemical energy; it is equal to the difference between the rate at which the plants in an ecosystem produce useful chemical energy (GPP) and the rate at which they use some of that energy

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during respiration. Some net primary production goes towards growth and reproduction of primary producers, while some is consumed by herbivores. Both gross and net primary production are in units of mass/area/time. In terrestrial ecosystems, mass of carbon per unit area per year (g C $m^{-2}year^{-1}$) is most often used as the unit of measurement.

3 Terrestrial Production

On the land, almost all primary production is now performed by vascular plants, with a small fraction coming from algae and nonvascular plants such as mosses and liverworts. Before the evolution of vascular plants, nonvascular plants likely play a more significant role. Primary production on land is a function of many factors but principally local hydrology and temperature. While plants cover much of the Earth's surface, they are strongly curtailed wherever temperatures are too extreme or where necessary plant resources (principally water and light) are limiting, such as deserts or polar regions.

Water is 'consumed' in plants by the processes of photosynthesis and transpiration. The latter process is driven by the evaporation of water from the leaves of plants. Transpiration allows plants to transport water and mineral nutrients from the soil to growth regions and also cools the plant. Diffusion of water out of a leaf is the force that drives transpiration and is regulated by structures known as stomata. These also regulate the diffusion of carbon dioxide from the atmosphere into the leaf, such that decreasing water loss also decreases carbon dioxide gain. Certain plants use alternative forms of photosynthesis, called crassulacean acid metabolism (CAM) and C4. These employ physiological and anatomical adaptations to increase water-use efficiency and allow increased primary production to take place under conditions that would normally limit carbon fixation by C3 plants.

4 Oceanic Production

In a reversal of the pattern on land, in the oceans, almost all primary production is performed by algae, with a small fraction contributed by vascular plants and other groups. Algae encompass a diverse range of organisms, ranging from single floating cells to attached seaweeds. They include photoautotrophs from a variety of groups. Eubacteria are important photosynthesizers in both oceanic and terrestrial ecosystems, and while some archaea are phototrophic, none are known to utilise oxygen-evolving photosynthesis (Schäfer et al. 1999). A number of eukaryotes are significant contributors to primary production in the ocean, including green algae, brown algae and red algae, and a diverse group of unicellular groups. Vascular plants are also represented in the ocean by groups such as the seagrasses. Unlike terrestrial ecosystems, the majority of primary production in the ocean is performed by free-living microscopic organisms called phytoplankton. Larger autotrophs, such as the seagrasses and macroalgae (seaweeds), are generally confined to the littoral zone and adjacent shallow waters, where they can attach to the underlying substrate but still be within the photic zone. There are exceptions, such as *Sargassum*, but the vast majority of free-floating production takes place within microscopic organisms.

The factors limiting primary production in the ocean are also very different from those on land. The availability of water, obviously, is not an issue. Similarly, temperature, while affecting metabolic rates, ranges less widely in the ocean than on land because the heat capacity of seawater buffers temperature changes and the formation of sea ice insulates it at lower temperatures. However, the availability of light, the source of energy for photosynthesis, and mineral nutrients, the building blocks for new growth, plays crucial roles in regulating primary production in the ocean.

4.1 Light

The sunlit zone of the ocean is called the photic zone (or euphotic zone). This is a relatively thin layer (10–100 m) near the ocean's surface where there is sufficient light for photosynthesis to occur. For practical purposes, the thickness of the photic zone is typically defined by the depth

at which light reaches 1% of its surface value. Light is attenuated down the water column by its absorption or scattering by the water itself and by dissolved or particulate material within it (including phytoplankton).

Net photosynthesis in the water column is determined by the interaction between the photic zone and the mixed layer. Turbulent mixing by wind energy at the ocean's surface homogenises the water column vertically until the turbulence dissipates. The deeper the mixed layer, the lower the average amount of light intercepted by phytoplankton within it. The mixed layer can vary from being shallower than the photic zone to being much deeper than the photic zone. When it is much deeper than the photic zone, this results in phytoplankton spending too much time in the dark for net growth to occur. The maximum depth of the mixed layer in which net growth can occur is called the critical depth. As long as there are adequate nutrients available, net primary production occurs whenever the mixed layer is shallower than the critical depth. Both the magnitude of wind mixing and the availability of light at the ocean's surface are affected across a range of space- and timescales. The most characteristic of these is the seasonal cycle, although wind magnitudes additionally have strong spatial components. Consequently, primary production in temperate regions such as the North Atlantic is highly seasonal, varying with both incident light at the water's surface (reduced in winter) and the degree of mixing (increased in winter). In tropical regions, such as the gyres in the middle of the major basins, light may only vary slightly across the year, and mixing may only occur episodically, such as during large storms or hurricanes.

4.1.1 Nutrients

Mixing also plays an important role in the limitation of primary production by nutrients. Inorganic nutrients, such as nitrate, phosphate and silicic acid, are necessary for phytoplankton to synthesise their cells and cellular machinery. Because of gravitational sinking of particulate material (such as plankton, dead or faecal material), nutrients are constantly lost from the photic zone and are only replenished by mixing or upwelling of deeper water. This is exacerbated where summertime solar heating and reduced winds increase vertical stratification and lead to a strong thermocline, since this makes it more difficult for wind mixing to entrain deeper water. Consequently, between mixing events, primary production (and the resulting processes that lead to sinking particulate material) constantly acts to consume nutrients in the mixed layer, and in many regions, this leads to nutrient exhaustion and decreased mixed layer production in the summer (even in the presence of abundant light). However, as long as the photic zone is deep enough, primary production may continue below the mixed layer where light-limited growth rates mean that nutrients are often more abundant.

Iron

Another factor relatively recently discovered to play a significant role in oceanic primary production is the micronutrient iron (Martin and Fitzwater 1988). This is used as a cofactor in enzymes involved in processes such as nitrate reduction and nitrogen fixation. A major source of iron to the oceans is dust from the Earth's deserts, picked up and delivered by the wind as Aeolian dust. In regions of the ocean that are distant from deserts or that are not reached by dust-carrying winds, the lack of iron can severely limit the amount of primary production that can occur. These areas are sometimes known as HNLC (high-nutrient, low-chlorophyll) regions because the scarcity of iron both limits phytoplankton growth and leaves a surplus of other nutrients. It is suggested introducing iron to these areas as a means of increasing primary productivity and sequestering carbon dioxide from the atmosphere (Cooper et al. 1996).

5 Measurement

The methods for measurement of primary production vary depending on whether gross versus net production is the desired measure and

whether terrestrial or aquatic systems are the focus. Gross production is almost always harder to measure than net because of respiration, which is a continuous and ongoing process that consumes some of the products of primary production before they can be accurately measured. Also, terrestrial ecosystems are generally more difficult because a substantial proportion of total productivity is shunted to below ground organs and tissues, where it is logistically difficult to measure. Shallow water aquatic systems can also face this problem.

Scale also greatly affects measurement techniques. The rate of carbon assimilation in plant tissues, organs, whole plants or plankton samples can be quantified by biochemically based techniques, but these techniques are decidedly inappropriate for large-scale terrestrial field situations. There, net primary production is almost always the desired variable, and estimation techniques involve various methods of estimating dry-weight biomass changes over time. Biomass estimates are often converted to an energy measure, such as kilocalories, by an empirically determined conversion factor.

6 Terrestrial

In terrestrial ecosystems, researchers generally measure net primary production. Although its definition is straightforward, field measurements used to estimate productivity vary according to investigator and biome. Field estimates rarely account for below ground productivity, herbivore, decomposition, turnover, litterfall, volatile organic compounds, root exudates and allocation to symbiotic microorganisms. Biomass-based NPP estimates result in underestimation of NPP due to incomplete accounting of these components (Clark et al. 2001; Scurlock et al. 2002). However, many field measurements correlate well to NPP. There are a number of comprehensive reviews of the field methods used to estimate NPP (Clark et al. 2001; Scurlock et al. 2002; Leith and Whittaker 1975). Estimates of ecosystem respiration, the total carbon dioxide produced by the ecosystem, can also be made with gas flux measurements.

The major unaccounted pool is below ground productivity, especially production and turnover of roots. Below ground components of NPP are difficult to measure. BNPP is often estimated based on a ratio of ANPP:BNPP rather than direct measurements.

7 Grasslands

Most frequently, peak standing biomass is assumed to measure NPP. In systems with persistent standing litter, live biomass is commonly reported. Measures of peak biomass are more reliable in if the system is predominantly annuals. However, perennial measurements can be reliable if there was a synchronous phenology driven by a strong seasonal climate. These methods may underestimate ANPP in grasslands by as much as twofold (temperate) to fourfold (tropical) (Scurlock et al. 2002). Repeated measures of standing live and dead biomass provide more accurate estimates of all grasslands, particularly those with large turnover, rapid decomposition and interspecific variation in timing of peak biomass. Wetland productivity (marshes and fens) is similarly measured. In Europe, annual mowing makes the annual biomass increment of wetlands evident.

8 Forests

Methods used to measure forest productivity are more diverse than those of grasslands. Biomass increment based on stand-specific allometry plus litterfall is considered a suitable although incomplete accounting of above-ground net primary production (ANPP). Field measurements used as a proxy for ANPP include annual litterfall, diameter or basal area increment (DBH or BAI) and volume increment.

9 Aquatic

In aquatic systems, primary production is typically measured using one of five main techniques (Marra 2002): (i) By variations in oxygen concentration within a sealed bottle. (ii) By incorporation of inorganic carbon-14 (^{14}C in the form of sodium bicarbonate) into organic matter (Steeman-Nielsen 1951, 1952). (iii) By stable isotopes of oxygen (^{16}O, ^{18}O and ^{17}O) (Bender et al. 1987; Luz and Barkan 2000). (iv) Fluorescence kinetics. (v) Stable isotopes of carbon (^{12}C and ^{13}C) (Luz and Barkan 2000).

The technique developed by Gaarder and Gran uses variations in the concentration of oxygen under different experimental conditions to infer gross primary production. Typically, three identical transparent vessels are filled with sample water and stoppered. The first is analysed immediately and used to determine the initial oxygen concentration; usually, this is done by performing a Winkler titration. The other two vessels are incubated, one under light and the other darkened. After a fixed period of time, the experiment ends, and the oxygen concentration in both vessels is measured. As photosynthesis has not taken place in the dark vessel, it provides a measure of ecosystem respiration. The light vessel permits both photosynthesis and respiration and so provides a measure of net photosynthesis (i.e. oxygen production via photosynthesis subtract oxygen consumption by respiration). Gross primary production is then obtained by adding oxygen consumption in the dark vessel to net oxygen production in the light vessel.

The technique of using ^{14}C incorporation (added as labelled Na_2CO_3) to infer primary production is most commonly used today because it is sensitive and can be used in all ocean environments. As ^{14}C is radioactive (via beta decay), it is relatively straightforward to measure its incorporation in organic material using devices such as scintillation counters.

Depending upon the incubation time chosen, net or gross primary production can be estimated. Gross primary production is best estimated using relatively short incubation times (1 h or less), since the loss of incorporated ^{14}C (by respiration and organic material excretion/exudation) will be more limited. Net primary production is the fraction of gross production remaining after these loss processes have consumed some of the fixed carbon.

Loss processes can range between 10 and 60% of incorporated ^{14}C according to the incubation period, ambient environmental conditions (especially temperature) and the experimental species used. Aside from those caused by the physiology of the experimental subject itself, potential losses due to the activity of consumers also need to be considered. This is particularly true in experiments making use of natural assemblages of microscopic autotrophs, where it is not possible to isolate them from their consumers.

10 Global

As primary production in the biosphere is an important part of the carbon cycle, estimating it at the global scale is important in Earth system science. However, quantifying primary production at this scale is difficult because of the range of habitats on Earth and because of the impact of weather events (availability of sunlight, water) on its variability.

Using satellite-derived estimates of the Normalized Difference Vegetation Index (NDVI) for terrestrial habitats and sea-surface chlorophyll for the oceans, it is estimated that the total (photoautotrophic) primary production for the Earth was 104.9 Gt C $year^{-1}$. Of this, 56.4 Gt C $year^{-1}$ (53.8%) was the product of terrestrial organisms, while the remaining 48.5 Gt C $year^{-1}$ was accounted for by oceanic production.

In arial terms, it was estimated that land production was approximately 426 g C m^{-2} $year^{-1}$ (excluding areas with permanent ice cover), while that for the oceans, was 140 g C m^{-2} $year^{-1}$ (Field et al. 1998). Another significant difference between the land and the oceans lies in their standing stocks—while accounting

for almost half of total production, oceanic autotrophs only account for about 0.2% of the total biomass.

11 Human Impact and Appropriation

Extensive human land use results in various levels of impact on actual NPP (NPP_{act}). In some regions, such as the Nile valley, irrigation has resulted in a considerable increase in primary production. However, these regions are exceptions to the rule, and in general, there is an NPP reduction due to land changes (ΔNPP_{LC}) of 9.6% across global land mass (Haberl et al. 2007). In addition to this, end consumption by people raises the total human appropriation of net primary production (HANPP) (Vitousek et al. 1986) to 23.8% of potential vegetation (NPP_0) (Haberl et al. 2007). It is estimated that, in 2000, 34% of the Earth's ice-free land area (12% cropland; 22% pasture) was devoted to human agriculture (Ramankutty et al. 2008). This disproportionate amount reduces the energy available to other species, having a marked impact on biodiversity, flows of carbon, water and energy and ecosystem services, and scientists have questioned how large this fraction can be before these services begin to break down (Foley et al. 2007).

12 Autotrophs Versus Heterotrophs

It is known that some organisms are capable of synthesising organic molecules from inorganic precursors and of storing biochemical energy in the process. These are called autotrophs, meaning 'self-feeding'. Autotrophs also are referred to as primary producers. Organisms able to manufacture complex organic molecules from simple inorganic compounds (water, CO_2, nutrients) include plants, some protists and some bacteria. The process by which they do this usually is photosynthesis, and as its name implies, photosynthesis requires light. Some producer organisms, mostly specialised bacteria, can convert inorganic nutrients to organic compounds without the presence of sunlight. There are several groups of chemosynthetic bacteria in marine and freshwater environments, particularly those rich in sulphur or hydrogen sulphide gas. Like chlorophyll-bearing plants and other organisms capable of photosynthesis, chemosynthetic organisms are autotrophs. Many organisms can only obtain their energy by feeding on other organisms. These are called heterotrophs. They include consumers of any organism, in any form: plants, animals, microbes and even dead tissue. Heterotrophs also are called consumers.

13 The Process of Primary Production

The general term 'production' is the creation of new organic matter. When a crop of wheat grows, new organic matter is created by the process of photosynthesis, which converts light energy into energy stored in chemical bonds within plant tissue. This energy fuels the metabolic machinery of the plant. New compounds and structures are synthesised, cells divide and the plant grows in size over time.

Whether one measures the rate at which photosynthesis occurs or the rate at which the individual plant increases in mass, one is concerned with primary production. The core idea is that new chemical compounds and new plant tissue are produced. Over time, primary production results in the addition of new plant biomass to the system. Consumers derive their energy from primary producers, either directly (herbivores, some detritivores) or indirectly (predators, other detritivores).

13.1 Energy Production by Primary Producers

The intensity of solar radiation reaching the Earth's surface depends partly on location: The maximum energy intensity is received at the equator, and the intensity decreases as one

moves towards the poles. The ecosystems have profound effects on climate, and this leads to the observed geographic patterns of biomes. Furthermore, only a small fraction of the Sun's radiation is actually used in the photosynthetic reaction in plants at the Earth's surface. Of the total solar radiation striking the Earth's outer atmosphere, about half of it is reflected back to space by ice, snow, oceans or deserts or absorbed by gases in the atmosphere—for example, the atmosphere's ozone gas layer absorbs nearly all ultraviolet light, which makes up about 9% of the Sun's radiation.

About half of the light that reaches Earth's surface is in the wavelength range that can be used by plants in photosynthesis (~400–700 nm wavelength)—this is called the photosynthetically active radiation, or PAR. Plants strongly absorb light of blue and red wavelengths; hence, they appear green in colour, the result of reflection of green wavelengths, as well as light in the far-infrared region, and they reflect light in the near-infrared region. Even if the wavelength is correct, the light energy is not at all converted into carbon by photosynthesis. Some of the light misses the leaf chloroplast, where the photosynthetic reactions occur, and much of the energy from light that is converted by photosynthesis to carbon compounds is used up in keeping the plant biochemical 'machinery' operating properly—this loss is generally termed 'respiration', although it also includes thermodynamic losses. Plants do not use all of the light energy theoretically available to them. On an average, plant gross primary production on Earth is about 5.83×10^6 cal m^{-2} $year^{-1}$. This is about 0.06% of the amount of solar energy falling per square metre on the outer edge of the Earth's atmosphere per year (defined as the solar constant and equal to 1.05×10^{10} cal m^{-2} $year^{-1}$). After the costs of respiration, plant net primary production is reduced to 4.95×10^6 cal m^{-2} $year^{-1}$ or about 0.05% of the solar constant. This is the 'average' efficiency, and in land plants, this value can reach ~2–3%, and in aquatic systems, this value can reach ~1%. This relatively low efficiency of conversion of solar energy into energy in carbon compounds sets the overall amount of energy available to heterotrophs at all other trophic levels.

13.1.1 Measuring Primary Production

There are two general approaches by which primary production can be measured: one can measure either (a) the rate of photosynthesis or (b) the rate of increase in plant biomass.

(a) *Rate of Photosynthesis*

$$6CO_2 + 6H_2O + \text{sunlight} \rightarrow C_6H_{12}O_6 + 6O_2.$$

If the plants are placed in a closed system and the depletion of CO_2 per unit time is measured or the generation of O_2, then a direct measure of primary production can be measured. The method which is used in studies of aquatic primary production illustrates this method well. In the surface waters of lakes and oceans, plants are mainly unicellular algae, and most consumers are microscopic crustaceans and protozoans. Both the producers and consumers are very small, and they are easily contained in a litre of water. If one puts these organisms in a bottle and turn on the lights, one gets photosynthesis. If one turns off the lights, then the primary production is shut down. However, darkness has no effect on respiration. Cellular respiration is the reverse process from photosynthesis.

$$C_6H_{12}O_2 \rightarrow 6CO_2 + 6H_2O.$$

Photosynthesis stores energy, and respiration releases it for use in functions such as reproduction and basic maintenance. When calculating the amount of energy that a plant stores as biomass, which is then available to heterotrophs, one must subtract plant respiration costs from the total primary production.

The general procedure is so simple that primary production of the world's oceans has been mapped in considerable detail and many of the world's freshwater lakes have also been investigated. One takes a series of small glass bottles with stoppers, and half of them are wrapped with some material such as tinfoil so that no light penetrates. These are called the 'light' and 'dark' bottles, respectively.

The bottles are filled with water taken from a particular place and depth; this water contains the tiny plants and animals of the aquatic ecosystem.

The bottles are closed with stoppers to prevent any exchange of gases or organisms with the surrounding water, and then they are suspended for a few hours at the same depth from which the water was originally taken. Inside the bottles, CO_2 is being consumed and O_2 is being produced, and we can measure the change over time in either one of these gases. For example, the amount of oxygen dissolved in water can be measured easily by chemical titration. Before suspending the bottles, the initial O_2 concentration is determined and expressed as mg of O_2 per litre of water (mg L^{-1}). Then, the final value is measured in both the light and dark bottles after a timed duration of incubation. What processes are taking place in each bottle that might alter the original O_2 or CO_2 concentrations. The equations below describe them:

Light bottle: In the light bottle, there is photosynthesis, or gross primary production (GPP), and there is respiration (R). The difference between these two processes, as we saw above, is net primary production = NPP = (GPP − *R*).
Dark bottle: In the dark bottle, there is no photosynthesis and only respiration.

The following simple example illustrates how we account for changes from the initial oxygen concentrations in the water that occurred during the incubation. Here, incubation period is 1 h. Measured oxygen concentrations are

$$\text{Initial bottle} = 8\,\text{mg}\,O_2\,L^{-1};$$
$$\text{Light bottle} = 10\,\text{mg}\,O_2\,L^{-1};$$
$$\text{Dark bottle} = 5\,\text{mg}\,O_2\,L^{-1}.$$

The oxygen increased in the light bottle compared to the initial due to photosynthesis, and the oxygen decreased in the dark bottle due to respiration. With this information, one can calculate the respiration, NPP and GPP for our system:

$$(\text{Light} - \text{Initial}) = (10 - 8) = 2\,\text{mg/L/h} = (\text{GPP} - R) = \text{NPP},$$
$$(\text{Initial} - \text{Dark}) = (8 - 5) = 3\,\text{mg/L/h} = \text{Respiration},$$
$$(\text{Light} - \text{Dark}) = (10 - 5) = 5\,\text{mg/L/h} = (\text{NPP} + R) = \text{GPP}.$$

Thus, the net and gross primary production as well as the respiration of our system can be measured. The oxygen technique is limited in situations where the primary production is very low. In these situations, the radioactive form of carbon, C^{14} ($^{14}CO_2$), can be used to monitor carbon uptake and fixation. The results could be converted between the oxygen and carbon methods by multiplying the oxygen values by 0.375 to put them into carbon equivalents (the factor comes from differences in atomic mass).

(b) *Rate of Biomass Accumulation*

Since the plants cannot be accumulated in bottles, therefore the following example is considered if one wants to know the primary production of a corn crop. Some seeds are planted, and at the end of 1 year, samples of the entire plants are harvested including the roots that were contained in 1 m^2 of area. These are dried to remove any variation in water content and then weighed to get the 'dry weight'. Thus, our measure of primary production would be g m^{-2} $year^{-1}$ of stems, leaves, roots, flowers and fruits minus the mass of the seeds that may have blown away.

Then what has been measured is not GPP, because some of the energy produced by photosynthesis is utilized to meet the metabolic needs of the corn plants themselves. Then is it NPP. If all the consumers are excluded such as insects of the corn plant, we would have a measure of NPP. But we assume that some insects and soil arthropods took a share of the plant biomass, and since we did not measure that share, we actually have measured something less than NPP. The same situation arises in the bottle method if we describe small heterotrophs that grazed on algae were included in the bottle, in which case the two methods would measure the same thing.

In recent years, it has also become possible to estimate GPP and R in large plants or entire forests using tracers and gas exchange techniques. These measurements now form the basis to measure primary production which affects the carbon dioxide content of our atmosphere.

Production, Standing Crop and Turnover

With either of these methods, the primary production can be expressed as the rate of formation of new material, per unit of Earth's surface, per unit of time. The production is reported as cal m^{-2} $year^{-1}$ (energy) or g m^{-2} $year^{-1}$ (dry organic matter).

Standing crop, on the other hand, is a measure of the biomass of the system at a single point in time and is measured as calories or grams per m^2. The ratio of the standing crop to the production (standing crop/production) is equal to the turnover of the system. By dividing standing crop (units of g m^{-2}) by production (units of g m^{-2} $year^{-1}$), one can see that the turnover is in units of 1/(1/year) = year in this example. Thus, the stock or standing crop of any material divided by the rate of production gives measure of time. It is really important to consider this element of 'time' whenever we are thinking about almost any aspect of an organism or an ecosystem. Learning about how much of something is happening and how fast it is changing is a critical aspect of understanding the system well enough to make decisions, for example, the decision of the forester above may be driven by economic concerns or by conservation concerns, but the 'best' choice for either of those concerns still depends on an understanding of the production, standing crop and turnover of the forest.

14 Patterns and Controls of Primary Production in the World's Ecosystems

In terms of NPP per unit area, the most productive systems are estuaries, swamps and marshes, tropical rain forests and temperate rain forests. If one wants to know the total amount of NPP in the world, one must multiply these values by the area that the various ecosystems occupied. In doing that, we find that now the most productive systems are open oceans, tropical rain forests, savannas and tropical seasonal forests. Basically, the answer is that climate and nutrients control primary productivity. Overall, the amount of water available limits land primary production on our world, in part due to the large areas of desert found on certain continents. Agricultural crops are especially productive due to 'artificial' subsidies of water and fertilisers, as well as the control of pests.

Even though temperature and especially precipitation are related to production, one notices a large degree of 'scatter' around the line of best fit. This scatter or variation is due in part to other aspects of particular (local) systems, such as their nutrient availability or their turnover rates. For example, grasslands can have a relatively high rate of primary production occurring during a brief growing season, yet the standing crop biomass is never very great. This is indicative of a high turnover rate. In a forest, on the other hand, the standing crop biomass of above-ground wood and below ground roots is large. Each year's production of new plant matter is a small fraction of total standing crop, and so the turnover of forest biomass is much lower.

Another good example is seen in the oceans, where most of the primary production is concentrated in microscopic algae. Algae have short life cycles, multiply rapidly, do not generate much biomass relative to their numbers and are eaten rapidly by herbivores. At any given point in time, then, the standing crop of algae in an ocean is likely low, but the turnover rate can be high.

15 The Flow of Energy to Higher Trophic Levels

Without autotrophs, there would be no energy available to all other organisms that lack the capability of fixing light energy. However, the continual loss of energy due to metabolic activity puts limits on how much energy is available to higher trophic levels (this is explained by the second law of thermodynamics). One looks at how and where this energy moves through an ecosystem once it is incorporated into organic matter.

Most of us are now familiar with the concept of the trophic level. It is simply a feeding level, as often represented in a food chain or food web. Primary producers comprise the bottom trophic level, followed by primary consumers (herbivores), then secondary consumers (carnivores feeding on herbivores) and so on. When we talk of moving 'up' the food chain, we are speaking figuratively and mean that we move from plants to herbivores to carnivores. This does not take into account decomposers and detritivores (organisms that feed on dead organic matter), which make up their own highly important trophic pathways.

What happens to the NPP that is produced and then stored as plant biomass at the lowest trophic level? On average, it is consumed or decomposed. The equation for aerobic respiration is

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

In the process, metabolic work is done and energy in chemical bonds is converted to heat energy. If NPP was not consumed, it would pile up somewhere. Usually this does not happen, but during periods of Earth history such as the Carboniferous and Pennsylvanian, enormous amounts of NPP in excess of consumption accumulated in swamps. It was buried and compressed to form the coal and oil deposits that we mine today. When we burn these deposits (same chemical reaction as above except that there is greater energy produced), we release the energy to drive the machines of industry, and of course, the CO_2 goes into the atmosphere as a greenhouse gas. This is the situation that we have today, where the excess CO_2 from burning these deposits (past excess NPP) is going into the atmosphere and building up over time. An ecosystem is in 'steady state' ('equilibrium') where annual total respiration balances annual total GPP. As energy passes from trophic level to trophic level, the following rules apply: Firstly, only a fraction of the energy available at one trophic level is transferred to the next trophic level. The rule of thumb is 10%, but this is very approximate. Secondly, typically, the numbers and biomass of organisms decrease as one ascends the food chain.

15.1 An Example: The Fox and the Hare

To understand these rules, we must examine what happens to energy within a food chain. Suppose we have some amount of plant matter consumed by hares and the hares are in turn consumed by foxes. A hare (or a population of hares) ingests plant matter; we will call this ingestion. Part of this material is processed by the digestive system and used to make new cells or tissues, and this part is called assimilation. What cannot be assimilated, for example, maybe some parts of the plant stems or roots, exits the hare's body, and this is called excretion. Thus, we can make the following definition: assimilation = (ingestion − excretion). The efficiency of this process of assimilation varies in animals, ranging from 15 to 50% if the food is plant material and from 60 to 90% if the food is animal material.

The hare uses a significant fraction of the assimilated energy just being a hare—maintaining a high, constant body temperature, synthesising proteins and hopping about. This energy used (lost) is attributed to cellular respiration. The remainder goes into making more hare biomass by growth and reproduction (i.e. increasing the overall biomass of hares by creating offspring). The conversion of assimilated energy into new tissue is termed secondary production in consumers, and it is conceptually the same as the primary production or NPP of plants. In our example, the secondary production of the hare is the energy available to foxes who eat the hares for their needs. Clearly, because all the energy costs of hares engaged in normal metabolic activities, the energy available to foxes is much less than the energy available to hares.

Just as we calculate the assimilation efficiency in the same way, we can also calculate the net production efficiency for any organism. This efficiency is equal to the production divided by the assimilation for animals or the NPP divided by

the GPP for plants. The 'production' refers to growth plus reproduction. In equation form, the net production efficiency = (production/assimilation) or for plants = (NPP/GPP). These ratios measure the efficiency with which an organism converts assimilated energy into primary or secondary production.

These efficiencies vary among organisms, largely due to widely differing metabolic requirements. For instance, an average, vertebrates uses about 98% of assimilated energy for metabolism, leaving only 2% for growth and reproduction. On an average, invertebrates use only ~80% of assimilated energy for metabolism and thus exhibit greater net production efficiency (~20%) than do vertebrates. Plants have the greatest net production efficiencies, which range from 30 to 85%. The reason that some organisms have such low net production efficiencies is that they are homeotherms, or animals that maintain a constant internal body temperature. This requires much more energy than is used by poikilotherms, which are organisms that do not regulate their temperatures internally.

Just as one can build our understanding of a system from the individual to the population to the community, in the same way, one can examine whole trophic levels by calculating ecological efficiencies. Ecological efficiency is defined as the energy supply available to trophic level N + 1, divided by the energy consumed by trophic level N. It can be considered as the efficiency of hares at converting plants into fox food. For example, the ecological efficiency = fox production/hare production. The ecological efficiency is a 'combined' measure that takes into account both the assimilation and net production efficiencies. This can be combined to different species of plants and animals into a single trophic level and then the ecological efficiency can be examined, for example, all of the plants in a field being fed on all of the different grazers from insects to cows.

Thinking about the overall ecological efficiency in a system brings us back to our first rule for the transfer of energy through trophic levels and up the food chain. In general, only about 10% of the energy consumed by one level is available to the next. For example, if hares consumed 1,000 kcal of plant energy, they might only be able to form 100 kcal of new hare tissue. For the hare population to be in steady state (neither increasing nor decreasing), each year's consumption of hares by foxes should roughly equal each year's production of new hare biomass. So the foxes consume about 100 kcal of hare biomass and convert perhaps 10 kcal into new fox biomass. In fact, this ecological efficiency is quite variable, with homeotherms averaging 1–5% and poikilotherms averaging 5–15%. The overall loss of energy from lower to higher trophic levels is important in setting the absolute number of trophic levels that any ecosystem can contain.

16 Pyramids of Biomass, Energy and Numbers

A pyramid of biomass is a representation of the amount of energy contained in biomass, at different trophic levels for a given point in time. The amount of energy available to one trophic level is limited by the amount stored by the level below. Because energy is lost in the transfer from one level to the next, there is successively less total energy as one moves up trophic levels. In general, one expects that higher trophic levels would have less total biomass than those below because less energy is available to them.

16.1 Pyramid of Numbers

Pyramid of numbers could also be constructed, which as its name implies represents the number of organisms in each trophic level. For the oceans, the bottom level would be quite large, due to the enormous number of small algae. For other ecosystems, the pyramid of numbers might be inverted, for instance, if a forest's plant community was composed of only a handful of very large trees, and yet there were many millions of insect grazers which are the plant material.

Just as with the inverted pyramid of numbers, in some rare exceptions, there could be an

inverted pyramid of biomass, where the biomass of the lower trophic level is less than the biomass of the next higher trophic level. The oceans are such an exception because at any point in time, the total amount of biomass in microscopic algae is small. Thus, a pyramid of biomass for the oceans can appear inverted. If the amount of energy in biomass at one level sets the limit of energy in biomass at the next level, as was the case with the hares and foxes, then how can one have less energy at the lower trophic level? Even though the biomass may be small, the rate at which new biomass is produced may be very large. Thus, over time, it is the amount of new biomass that is produced, from whatever the standing stock of biomass might be, that is important for the next trophic level.

This further can be examined by constructing a pyramid of energy, which shows rates of production rather than standing crop. Algal populations can double in a few days, whereas the zooplankton that feed on them reproduce more slowly and might double in numbers in a few months, and the fish feeding on zooplankton might only reproduce once a year. Thus, a pyramid of energy takes into account the turnover rate of the organisms and can never be inverted. Thinking about pyramids of energy and turnover time is similar to the residence time of elements. But here, one is talking about the residence time of 'energy'. The residence time of energy is equal to the energy in biomass divided by the net productivity, R_t = (energy in biomass/net productivity). The residence time of energy in the primary producers of various ecosystems is calculated; then we find that the residence times range from about 20 to 25 years for forests (both tropical rainforests and boreal forests), down to ~3–5 years for grasslands and finally down to only 10–15 days for lakes and oceans. This difference in residence time between aquatic and terrestrial ecosystems is reflected in the pyramids of biomass. It is also very important to consider in analysing how these different ecosystems would respond to a disturbance or what scheme might best be used to manage the resources of the ecosystem.

17 Humans and Energy Consumption

All of the animal species on Earth are consumers, and they depend upon producer organisms for their food. For all practical purposes, it is the products of terrestrial plant productivity that sustain humans. Then one can start by looking at the inputs and outputs:

Inputs: NPP, calculated as annual harvest. In a cropland, NPP and annual harvest occur in the same year. In forests, annual harvest can exceed annual NPP (e.g. when a forest is cut down, the harvest is of many years of growth), but we can still compute annual averages (Amthor and Baldocchi 2001).

Outputs: Total productivity of lands devoted entirely to human activities. This includes total cropland NPP and also energy consumed in setting fires to clear land. A high estimate is obtained by including lost productive capacity resulting in converting open land to cities and forests to pastures and due to desertification and other overuse of land. This is an estimate of the total human impact on terrestrial productivity.

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Aquatic Weeds: Occurrence and Distribution

2

1 Introduction

The unwanted plants that grow in the wetland are termed as aquatic weeds. They generally grow in the fields and compete with crops for water, soil nutrients, light and space, and crop yields are reduced. They also harbour insect pests and microorganism. The inhibitors or poisonous substances released into soil by certain weeds are harmful to the plants, human beings and livestocks. They block the drainage and impede the flow of water in canals and water-transport channels, and their growth in the rivers makes navigation very difficult. The water pollution is increased by the dense growth of the weeds in water because they deoxygenate the water and kill the fish.

2 Natural Powers of Weeds

Weed seeds are likely to germinate earlier, their seedlings grow faster, they flower earlier and form seeds in profusion and maturation is earlier than the crop they infest. Nature has bestowed these qualities on weeds so that there seeds are collected unwarily along with the produce of the crop at harvest and get distributed to other places where the produce may be taken. They have the remarkable capacity to germinate under varied conditions, but very characteristically, they are season-bound and the peak period of germination always takes place in certain seasons in regular succession year after year. Another characteristic of the weed seeds is the possession by them of the phenomenon of dormancy which is an intrinsic physiological power of the seed to resist germination even under favourable conditions, and also the seeds do not lose their viability for years even under adverse conditions.

3 Types of Weeds

Weeds belong to the class Angiospermae (flowering plants) which have two subclasses: Monocotyledoneae (monocots) and Dicotyledoneae (dicots). On the basis of the habitats, they are divided into terrestrial and aquatic categories, and on the basis of the duration of life, they are divided into annuals, biennials and perennials. Annuals live and produce their seeds in a single growing season. Biennials need two growing seasons; in one season, they pass through their vegetative or rosette stage, followed by reproductive stage in the next season. The multiplication of both the annuals and the biennials is through seed. Perennials live indefinitely and are propagated not only through seeds but often vegetatively through underground structures, such as rhizomes, stolons, bulbs and tubers. Perennials are of two types: the simple and the creeping. The former multiply only through seeds. They have no normal means of spreading vegetatively. However, if they are injured or cut, the severed portion produces new plants. Creeping perennials are spread by creeping

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roots, creeping above-ground stems and creeping underground stems. Some weeds propagate themselves by means of tubers which are modified rhizomes adapted for the storage of food.

Aquatic weeds are classified into three types, namely, submerged, emersed and floating. Submerged aquatics are anchored to the bottom of the habitats, for example, a ditch, can grow entirely beneath the surface of the water. Emersed ones have their roots beneath the surface of the water, but the leaves and stems are above the waterline. Floating weeds or surfaced aquatics either float freely on the water or float only in a limited area. There is still another group of weed known as the parasitic weeds. These are of two kinds: the total parasite and the partial parasites. These weeds parasitize certain host plants, which they directly attack, and deprive them of water, nutrients and assimilates.

4 Principles of Crop Production and Weed Destruction

4.1 Crop–Weed Competition

In nonirrigated areas, the competition between weeds and crops is largely for water. The transpiration coefficients for *kunda (Ischaemum pilosum), hariyali (Cynodon dactylon)* and *Tephrosia purpurea* are 556, 813 and 1,108, respectively, whereas this coefficient is only 430 for *jowar (Sorghum vulgare).* A saving of 750–1,250 ton of water per hectare of soil, forming a 1-m-deep column, is possible by keeping the soil free from weeds. In irrigated tracts, the competition is severe for nutrients. The unchecked growth of weeds, in a wheat fields measuring 1 ha, removes about 20 kg of nitrogen, reducing the grain yield by about 12 quintals. The mineral requirements of weeds are high. Wild mustard *(Brassica sinensis)* requires twice as much nitrogen, twice as much phosphorus, four times as much potash and four times as much water as a cultivated oat plant. Weeds in a fallow land deplete the soils of both moisture and nutrients. *Pohli (Carthamus oxyacantha)* removes about 60 kg of nitrogen from 1 ha.

4.2 The Identification of Weeds

Both the nature of the weeds and that of the crops which they infest influence the action of the weedicides. For example, the same types of weeds occur in maize and cotton which are *kharif* crops. 2,4-D can be used for killing the weeds in maize, but this weedicide should never be used on cotton which is highly sensitive to it. The successful application of chemicals to smother the weeds in growing crops is determined by the knowledge one has regarding the biology of both the crops and the weeds. The degree of success from the adoption of physical methods of weed removal is determined largely by the knowledge one has of the peak periods when the seeds of weeds are formed and of the nature or duration of their dormancy.

4.3 Preventive Measures

Nature has provided weeds with a number of devices that help them to be disseminated widely. The agencies that facilitate the dispersal of weed seeds far and wide are water, wind and animals, including man. The troubles that weeds create in crops, soil and water are summed up in the adage ‘one year of seedling is seven years of weeding’. To avoid such a situation, a wise step is to follow the principle ‘prevention is better than cure’.

As weed seeds are so readily dispersed by natural agencies and by the farmer himself, it is important to prevent weeds, whether in crops, on borders or bunds, in fences or in irrigation channels, from flowering and setting seed. Preventive methods consist in sowing crop seeds not contaminated with weed seeds, using manure and irrigation water not laden with them and the enforcement of weed control laws and seed-certification measures.

4.4 Control Methods

Measures against weeds comprise mechanical (cultivation and moving), cultural or cropping, biological and chemical means. Each of these methods has certain merits, and a prudent farmer can make use of one means or a combination of them to control weeds efficiently and economically.

4.4.1 Mechanical Methods

Hand-pulling or hand-weeding, hoeing, tilling, mowing, burning, flooding, smothering, etc. are examples of physical methods of weed control, involving the use of physical energy through implements, either manual, bullock-drawn or power-operated. Hand-weeding is the most efficient method, but it is back-breaking, time-consuming and costly. With the gradual industrialization of our country, coupled with the raising of standards of living and literacy, manual labour is becoming scarce. Tillage is a practical and economical method of controlling annual weeds. The plough, the harrow and the cultivator are implements in use to eradicate weeds.

4.4.2 Cultural or Cropping Methods

Weeds under many conditions are better competitors than crop plants for light, water, nutrients and soil space. However, farming practices are capable of changing the condition in such a way as to enable the crop plants to compete with weeds successfully or to reduce their interference to the minimum and thus preventing them from acting as impediments to increased crop production. Seeds with good germination will give the crop a vigorous and close stand and thus enable it to steal a march on the weeds. Varieties which are well adapted to a region will obviously compete better with the weeds than varieties poorly adapted to it. The plant breeder has to evolve quick-growing and short-duration varieties of crop plants with a large leaf area and good branching, and the agronomist has to work out the proper seed rate, depth, time and method of sowing and applying the most effective methods of irrigation and fertilisers and adopt a proper system of rotation. Some crops can compete better with weeds than others. For instance, crops like Sudan grass, sorghum and cowpea are good competitors, while crops, such as linseed, groundnut and lentil, are poor competitors. The raising of highly competitive crops is useful in reducing weed infestations. One disadvantage from which many smother crops suffer is that their seedlings grow slowly. If these crops are grown for seed, some measures have necessarily to be taken to control weeds in the early growth of these crops. However, if they are meant for fodder, the thick sowing resulting in dense growth smothers the weeds.

4.4.3 Chemical Methods

The controlling of weeds in the growing crops with weedicides increases their yields and ensures the efficient use of irrigation, fertilisers and plant-protection measures, such as the spraying of insecticides and fungicides. The removal of weeds from the growing crops facilitates easy harvesting and gives a high-quality produce without admixture with weed seeds. Chemical weed control can be adopted quite in time and in situations and under conditions which make manual or mechanical weeding difficult. A great advantage of this method lies in killing weeds in the crop row or in the immediate vicinity of crop plants. The chemical method is easier, less time-consuming and less costly than weeding by hired labourers.

4.5 Aquatic Applications

A number of chemicals are used for controlling some submerged aquatic weeds by dissolving or emulsifying them in water in canals, ditches, ponds and lakes. Some chemicals in use are aqualin, aromatic solvents, chlorinated benzenes, copper sulphate, endothall, Fenac, sodium arsenite and 2,4-D.

4.5.1 Formulation of Herbicides

Formulation refers to the way in which the basic weed-killing chemicals are prepared for practical

use. Herbicides are formulated to be applied as solutions in water or oil, emulsions, wettable powders, granules and dusts.

4.5.2 Solutions of Water or Oils

The salts of most herbicides are soluble in water. They are dissolved in convenient amounts of water and then sprayed. A few examples are sodium and amine salts of 2, 4-d, 2 and 4,5-T; MCPA; amine salts of DNBP; sodium salts of pentachlorophenol, TCA and dalapon. The 'parent acid' formulations of some of these are soluble in oil (DNBP, PCP). They are often used to increase the toxicity of oil sprays or to fortify the oil.

4.5.3 Emulsions

An emulsion is one liquid dispersed in another liquid, each maintaining its original identity. The two liquids are prevented from reacting with each other by the addition of an emulsifying agent. Ester formulations of 2,4-D are oil-like and form emulsions. Emulsions are milky.

4.5.4 Wettable Powder

It is a type of formulation in which a herbicide is absorbed generally on an inert carrier, together with an added surface-acting agent, and finely ground so that it will form a suspension when agitated with water. Simazine, atrazine, monuron, diuron and neburon are wettable powders.

4.5.5 Granular Herbicides

In these formulations, the herbicide is absorbed or, mixed with or impregnated into a generally inert carrier in such a way that the final product consists of granular particles. Many carriers are used, for example, clay, sand vermiculite and finely ground plant parts. Granular materials can be spread by hand or with mechanical spreaders. These materials have advantages over sprays, because water is not needed for application, costlier spraying equipment is dispensed with and the granules fall off the leaves of valuable plants without causing injury. Another advantage is that as the active weed-killing principle in these formulations is gradually released, they suppress the growth of weeds for long.

4.5.6 Dusts

Insecticides and fungicides are very often made in the form of dusts. However, only a few herbicides are applied as dusts because of the drift hazard.

4.6 Calculation of the Dosage Levels

The quantity of the toxic ingredients, to which the weed-killing property of a chemical is due, is different in different formulations of herbicides. The active ingredient is that part of a chemical formulation which is directly responsible for the herbicidal effects. In some herbicides, the entire molecule may be the active unit. If the chemical is 99% pure, it is considered to have 99% active ingredient. The strength of the wettable powders is expressed in terms of the active ingredient (a.i.). In others, the herbicidal activity is calculated on an acid-equivalent basis. The acid equivalent (a.e.) refers to that part of a formulation which can be theoretically attributed to the acid. In these cases, the acid equivalent is given as the active ingredient. Examples are 2, 4-D, MCPA, MCPB, 2,4-DB, CMPP and picloram.

5 Aquatic Weed Control

Unwanted aquatic plants are the number one pond maintenance problem. With the arrival of warm temperatures, they annoy pond owners and create problems throughout the summer and fall. Depending on the species and abundance of these weeds, it is often necessary to control them. Aside from the aesthetic value of a well-kept pond, an over abundance of weeds can create a hazard for aquatic life, offensive odours, breeding grounds for mosquitoes and a hindrance to water sports. There are three basic ways of controlling aquatic weeds, and these are mechanical, biological and chemical.

Mechanical is useful in the event it is a small pond and the weeds are minimal. One can pull out or dig out problem weeds. It is time-consuming and eventually impossible if let go.

Biological control (use of grass carp) can be successful if done before a problem occurs or after a chemical treatment has knocked down the problem. These fishes do not breed in standing water and only eat weeds. Restocking will be necessary periodically.

Chemical control requires specific weed identification and treatment as necessary with either an algaecide or herbicide.

There are five major groups of aquatic weeds:

Planktonic—(usually not a problem unless potable water), filamentous (slimy and green, or horsehair clump) and Chara (also called muskgrass, anchored in the bottom and underwater).

Marginal weeds—Plants that grow in the saturated soil on the water's edge, like cattails.

Submersed weeds—True seed plants rooted on the bottom, mostly underwater a few flowers above the surface like naiads.

Emersed weeds—Rooted on the bottom with floating leaves and flowers, like arrowhead and water willow.

Floating weeds—Free-floating plants or rooted weeds, but leaves rise and fall with water level, like duckweed and water lilies.

5.1 Damages Caused by Aquatic Weeds

Aquatic weeds are those unabated plants which grow and complete their life cycle in water and cause harm to aquatic environment directly and to related eco-environment relatively. Water is one of most important natural resource and in fact basis of all life forms on this planet. Therefore, appropriate management of water from source to its utilisation is necessary to sustain the normal function of life. It is one important part of natural resource management. The presence of excessive aquatic vegetation influences the management of water in natural waterways, man-made canals and reservoirs which amounts to millions of kilometres/square kilometres of such waterbodies around the world. The area under small tanks and ponds is equally important due to the establishment of many small irrigation schemes and watershed management projects all over the world. For example, India has 1.9 m ha underwater in reservoirs and 1.2 m ha under irrigation canals. The area under village ponds and tanks is nearly 2.2 m ha. Aquatic weeds often reduce the effectiveness of waterbodies for fish production. Aquatic weeds can assimilate large quantities of nutrients from the water reducing their availability for planktonic algae. They may also cause reduction in oxygen levels and present gaseous exchange with water resulting in adverse fish production. Although excessive weed growth may provide protective cover in water for small fish growth, it may also interfere with fish harvesting. Dense growth of aquatic weeds may provide ideal habitat for the development of mosquitoes causing malaria, encephalitis and filariasis. These weeds may also serve as vectors for disease-causing organisms and can greatly reduce the aesthetic value of waterbodies from a recreational point of view. Aquatic weeds have been found to severely reduce the flow capacity of irrigation canals, thereby reducing the availability of water to the farmers field. Aquatic weeds may also damage pumps and turbines in super thermal power stations and hydroelectric power stations influencing electric production and increasing the cost of maintenance of power stations. Many aquatic plants are desirable since they may play temporarily a beneficial role in reducing agricultural, domestic and industrial pollution. Many aquatic weeds may play a useful role of providing continuous supply of phytoplanktons and help fish production.

Some of the harmful effects of aquatic weeds are as follows:

5.2 Harmful Effects of Weeds

1. Reduces water storage capacity in reservoirs, tanks and ponds
2. Impedes flow and amount of water in canals and drainage systems
3. Reduces fish production
4. Interferes with navigation and aesthetic value
5. Promotes habitat for mosquitoes

Aquatic weeds (emergent, floating and submerged) interfere with the static and flow of

water system. They cause tremendous loss of water from waterbodies like lakes and dams through evapotranspiration. In flowing water system, aquatic weeds impede the flow of water in irrigation canals and drainage channels, thereby increasing evaporation damage structures in canals and dams, clog gates, siphons, valves, bridge piers, pump, etc. Impediment in flow of water may result in localised floods in neighbouring areas. India has the largest canal network in the world where the velocity of flowing water is reduced by about 30–40% due to the presence of aquatic weeds. Floating and deep-rooted submerged weeds interfere with navigation. Water hyacinth and Alligator weed grow profusely and create dense mats which prevent the movement of boats and at times even large ships. Village ponds and tanks get infested with floating and submerged weeds which results in reducing the capacity of the water storage and therefore effecting efficient irrigation. Therefore, considering the losses caused, it is essential to keep aquatic weeds under control in waterbodies, flow water systems, ponds and tanks so that these systems can be utilised to best of their efficiency.

Mechanical methods are being practised at present as use of chemicals is very much restricted due to the difficulty in control on water use for different purposes. Use of bioagents for weed control is under experimental dissemination and needs further research and refinement in technology for control of aquatic weeds. Within the next two decades, bioagents will be one of the major methods of controlling aquatic weeds, especially the floating ones. Research is also necessary for studying the various factors influencing the aquatic environment and the resultant vegetation. Researchers are envisaging to establish an integrated approach to aquatic weed control using a mix of mechanical and biocidal techniques to control aquatic weeds under specific situations.

6 Classification of Aquatic Weeds

Aquatic weeds are classified according to various habitats which form their eco-environment and become conducive for their growth, reproduction and dissemination.

6.1 Emergent Weeds

These weeds grow in shallow waters and situations existing near the waterbodies where water recedes and rises with the seasons or regular releases from a large waterbody or reservoir. Most of such situations are of permanent in nature where minimum and maximum water levels are consistent. Such situations include banks of canals, rivers, periphery of waterbodies which are mostly in earthen dams and partly in masonry dams, drainage ditches and water ponds near villages. These weeds may be called semi-aquatic but more appropriately referred to as emergent aquatic weeds. Some examples of the emergent weeds are given below:

Botanical name	Common name	Family
Typha angustata	Cattail narrow leaved	Typhaceae
T. latifolia	Cattail common	Typhaceae
T. orientalis	Cattail	Typhaceae
Phragmites communis Trin.	Common reed	Poaceae
P. karka	Common reed	Poaceae
P. australis	Common reed	Poaceae
Pontederia cordata L.	Pickrel weed	Pontederiaceae
Commelina benghalensis L.	Watergrass	Commelinaceae
Alisma plantago	Water cattail	Alismataceae
Cyperus difformis L.	Umbrella plant	Cyperaceae
Ipomoea carnea Jacq.	Besharam	Convolvulaceae

There are situations where vast areas of land remain inundated with water for long periods of time and may only dry out in severe drought conditions. Such lands are known as marshes or swampy areas. They support a different type of vegetation which may include plants/weeds that are capable of growing under both flooded and saturated conditions. These may include annuals

to large trees. Some of these amphibious species are given below:

Temporary water situations		
Botanical name	*Common name*	*Family*
Alternanthera philoxeroides (Mart.)	Griseb. Alligator weed	Amaranthaceae
Marsilea minuta L.	Pepper west	Marsileaceae
Heteranthera limosa (SW)	Wild mud plantain	Pontederiaceae
Monochoria vaginalis Presi.	Carpet weed	Pontederiaceae
Panicum purpurascens	Raddi. Paragrass	Poaceae
Paspalum fluitans Kunth.	Water paspalum	Poaceae
Clay substratum situation		
Botanical name	*Common name*	*Family*
Fimbristylis miliacea Vahl.	Hoorah grass	Cyperaceae
Floating mat situation		
Botanical name	*Common name*	*Family*
Ipomoea aquatica Forsk.	Floating morning glory	Convolvulaceae
Hydrocotyle umbellata L.	Water pennywort	Hydrocolylaceae
Jussiaea repens L.	Water primrose	Onagraceae
Ludwigia parviflora	Water purslane	Onagraceae
Trapa bispinosa Roxb.	Water chestnut	Trapaceae

6.2 Floating Weeds

These are plants which grow and complete their life cycle in water. They vary in size from single cell (algae) and may grow up to large vascular plants. In case of drying of waterbodies, most of them give their seeds and other vegetative reproductive organs in base ground lands. These weeds are observed in the surface of the large, deep and shallow depths of waterbodies; deep continuous flowing canals and continuously flowing rivers, large ponds, tanks, etc. Some of the weeds in this ecosystem freely float and move long distances, while some of them do float on the water surface but anchor down to soil at the bottom of the waterbody. These weed species make loss of water through evapotranspiration in addition to impediment caused in flow of water. Therefore, these weeds can be classified in two subgroups, namely, (a) free-floating and (b) rooted floating weeds. Examples of common weeds under each subgroup are given below:

(a) *Free-floating weeds*		
Botanical name	*Common name*	*Family*
Eichhornia crassipes (Mart.) Solens	Water hyacinth	Pontederiaceae
Salvinia auriculata (Mitch.) Syn.	Water fern	Salviniaceae
S. molesta	Water fern	Salviniaceae
S. natans	Water fern	Salviniaceae
Pistia stratiotes L.	Water lettuce	Araceae
Lemna minor	Duck weed	Lemnaceae
Spirodela polyrhiza (L.) Schlcid	Giant duck weed	Lemnaceae
Azolla imbricata waxai	Water velvet	Salviniaceae
A. pinnata	Water velvet	Salviniaceae
Polygonum amphibium L.	Water smart weed	Polygonaceae
(b) *Rooted floating weeds*		
Botanical name	*Common name*	*Family*
Sagittaria guayanensis HBK	Arrowhead	Alismataceae
Ipomoea hederacea	Nilkalmi	Convolvulaceae
Nelumbo nucifera	G Lotus	Nymphaceae
Nymphaea alba L.	White water lily	Nymphaceae
Nuphar lutea L.	Yellow water lily	—do—
Zannichellia palustris L.	Horned pondweed	Zannichelliaceae

6.3 Submerged Weeds

Weed species belonging to this group germinate/sprout, grow and reproduce beneath the water surface. Their roots and reproductive organs remain in the soil at the bottom of the waterbody. These weeds damage the maximum, because they are not visible on the surface and impede the flow of water varying upon the degree of their intensity and growth. Most of these weeds are

found in shallow and medium deep waterbodies and continuous flowing canals and drainage ditches. The ecosystem provides situations which allows the growth of algae, filamentous algae and higher algae in shallow water situations and under deep water situations, and thus, submerged weeds may be further categorised as (a) shallow water submerged weeds and (b) deep water submerged weeds. Commonly occurring weeds in these categories are as follows:

(a) ***Shallow water submerged weeds***		
Algae		
Botanical name	*Common name*	*Family*
Anabaena spp.	Blue-green algae	Nostocaceae
Cladophora spp.	Cotton mat-type algae	Cladophoraceae
Pithophora spp.	Wet wool-type algae	Chlorophyceae
Spirogyra spp.	Slimy green algae	Chlorophyceae
Chara zeylanica	Musk grass	Characeae
Nitella hyalina	Stonewort	Characeae
Higher plants		
Botanical name	*Common name*	*Family*
Najas minor	All Naiad	Najadaceae
Vallisneria spiralis	Eel weed	Najadaceae
Potamogeton crispus	Curly-leaf pondweed	Potamogetonaceae
P. natans L.	Broadleaved pondweed	—do—
P. pusillus L.	Small pondweed	—do—
P. nodosus	—do—	
P. pectinatus	—do—	
(b) ***Deep water-rooted submerged weeds***		
Botanical name	*Common name*	*Family*
Myriophyllum spicatum L.	Eurasian water milfoil	Haloragaceae
Hydrilla verticillata	Royle Hydrilla	Hydrocharitaceae
Elodea canadensis	Elodea	—do—
Utricularia flexuosa Vahl.	Bladderwort	Lentibulariaceae

7 Ecological Problems Related to Aquatic Environment

Ecologically, aquatic vegetation can be broadly grouped as algae and hydrophytes.

7.1 Algae

The fresh and saline water exposed to sunlight provide ideal ecological environment to algal growth. Majority of them are purely aquatic in nature and adapt to live in ponds, lakes, streams, swimming pools and oceans. Freshwater algae are grouped as planktonic and filamentous in nature. Planktonic algae may colour the water as green, yellow, red and black. They may physically look as scums or water bloom. Physiologically they use solar energy to convert it into food, use CO_2 from water during photosynthesis (in day) and produce oxygen as by-product. During dark hours, they release CO_2 through respiration and consume O_2. Some of the algae maintain balance in natural aquatic environments as they produce O_2 and provide food for most of the fish and other aquatic animals. Although under normal conditions algae are beneficial, their overpopulation may be undesirable for domestic and commercial water uses. Excessive phytoplanktonic booms may result to zooplanktonic developments which deplete oxygen and lead to eutrophication which may prove destructive to fish and other aquatic wildlife. Dense growth of planktonic algae will create shade, thus reducing light to the bottom of waterbody, and this may prevent germination of seeds, growth of rooted submerged weeds and may result in destabilisation of aquatic environment occurs. Generally, they do not disturb irrigation systems but may spoil the places of recreation value like swimming due to bad odours and scum created on water surface. Some of them may prove toxic to fish, birds and domestic animals. The filamentous algae consist of thread-like structures or filaments made of single cells attached end to end. They do not have roots, stems, etc. and grow in cool and warm waters. They cause

immense nuisance in irrigation system. The important genera include *Chara, Nitella, Spirogyra, Hydrodictyon, Cladophora and Pithophora.* The filamentous and planktonic algae produce undesirable odours and spoil the taste of drinking water, interfere with domestic and industrial usage and clog the filters of the water treatment plants. They also provide a coating to cooling towers and condensers. The filamentous algae clog the weirs and lining of the canals thereby interfere with the irrigation system. It may also adversely influence the fish production.

7.2 Hydrophytes

These plants grow fully or partly submerged in water. There are more than 100 families that represent vascular plants. These plants are structurally different from meso or xerophytes. They have less-developed protective and conductive tissues. They have extensive arrangement for buoyancy and aeration, particularly in ground tissue of petiole and leaf mesophyll and cortex of stem and root.

7.2.1 Emergent Weeds

Most of the weeds are of emergent nature which can grow under saturated and emerged soil-water condition. They grow in soil from saturated moisture on the banks of canals, depression in river shoreline and canals into water up to depth of 1 m. These weeds may vary in growth according to its habitat. Plants belong to grassy, broadleaved, shrubs categories. These plants are also found in marshy habitats from saturated to shallow submergence, low-lying stretches of lands which remain for a fixed time underwater. These weeds have been observed on the bank of irrigation canals and drainage ditches. They have been observed in seepage areas of canals, depressions containing water along canals, rivers flowing with slow velocity, earthen embankments of water reservoirs, tanks and shallow depths of water reservoirs. Most of these weeds have been observed in and around waterbodies in tropical and subtropical parts of the world as the ecological environments of these regions are highly congenial for growth, reproduction and dissemination of these weeds. The important genera to which most of the weed species belongs are *Typha, Polygonum*, *Phragmites, Alternanthera, Scirpus, Ipomoea, Tamarix, Cephalanthus, Populus, Juncus, Cyperus, Monochoria,* etc. Some of the important weed species observed are *Typha angustata, T. latifolia, Ipomoea carnea, Phragmites karka and Monochoria vaginalis.*

7.2.2 Floating Weeds

Many aquatic plants have leaves floating on water surface either singly or in groups. They have true roots, leaves and flowering parts above the water surface. Some of them are free floating, while the roots of few are anchored in mud in the bottom of waterbody. These plants rise and fall with the level of water in the waterbody. They flower very rapidly, and some of them are the most troublesome aquatic weed of the world. Free-floating weeds belong to the genera *Eichhornia, Pistia, Lemna, Salvinia, Nymphaea, Brasenia spirodela,* etc.

Eichhornia crassipes, Salvinia molesta and Pistia stratiotes are some of the most problematic weeds in the tropical and subtropical regions of the world. Surface weeds have a category where plants are rooted in the mud below the waterbody and leaves are at or above the water surface. These plants grow in situations where free water is available at ground level to a depth which nears half the depth of normal aquatic plant species. The plants may be broadleaved or narrow-like grasses. The leaves do not rise and fall with increase and decrease in water levels as in case of anchored floating weeds. Some of these weeds belong to genera *Nuphar*, *Nelumbo, Jussiaea, Myriophyllum, Sparganum, Pontederia, Sagittaria, Rorippa, Lytham, Epilobium,* etc.

Various ecological parameters influence the growth, reproduction and dissemination of the weeds. Variability in the depth of water, nutrient content in the water and contamination of water with industrial pollutants like metallic ions greatly influence the growth of these weeds. The source of contamination in flow water

system like rivers and canals and silting in case of anchored surface weeds greatly influence weed species and their growth. Velocity of water in rivers and canals greatly influences the growth and stability of these weeds. Greater presence of free-floating weeds has been observed in rivers and canals which have a slow water velocity.

7.2.3 Submerged Weeds

There are mostly vascular plants that produce most of its vegetation under the water surface. Most of these plants emerge from seeds and propagules and have true roots, stems and leaves. Abundance of these weeds is dependent on the depth and turbidity of water and physical characteristics at the bottom of the water surface. A depth of 3.5–4.0 m. in clear water is the reasonable limit of submerged weeds. They are capable of absorbing nutrients through leaves, stems and roots. Severe competition exists with planktonic alga for nutrients and results in decreased production. The submersed weeds belong to the genera *Potamogeton, Elodea, Myriophyllum*, *Ceratophyllum, Utricularia, Ranunculus, Heteranthera, Alisma, Zannichellia,* etc. Some of the important problem weeds include *Hydrilla verticillata*, *Potamogeton nodosus and P. pectinatus* (major problem in Chambal and Bhakra Nangal Command canals in India).

8 Effect of Aquatic Weeds on Environment

Aquatic weeds create number of environmental problems. They create situations which are ideal for mosquito growth. The mosquitoes are sheltered and protected from their predators by aquatic weed roots and leafy growth and are responsible for the spread of malaria, yellow fever, river blindness and encephalitis. Snails are able to multiply, playing a crucial role in the life cycle of blood and liver flukes (parasitic worms) as they shelter and find sustenance among the root zones. Schistosomiasis and fascioliasis diseases spread as the floating weed carries the snails to new locations. People living close to these areas complain of mosquito problems. Fish production is greatly affected by the presence of floating and submerged aquatic weeds. Isolated weed beds may be tolerated, providing shelter and shade for fish, but when the growth becomes thick and covers entire waterbody, it can be lethal for fish growth. Fish may suffocate from a lack of oxygen and may cause death. When floating and submerged aquatic weeds become extremely dense, many fish species are unable to exist in such environments and vanish. For example, fishes production in Harike lake in Punjab is decreasing and is a matter of concern to all. The decomposition of huge amounts of biological mass creates condition where CO_2 and carbon monoxide are produced and released to the atmosphere. The decomposition period is much less than decomposition of other vegetation on land. The decomposition creates emissions of foul smells which are unpleasant to public convenience. Mosquitoes and other parasites grow in these situations and affect the life of those living in close proximity.

Waterbodies which are places of recreational and aesthetic use are badly affected by unwanted growth of aquatic weeds. The decomposition of weed material consists of siliceous material and other insoluble salts which settle on the bottom of the waterbody. Dense weed growth slows the flow of water in rivers, canals and drainage ditches allowing silt to settle out and be deposited on the bed of the waterbody. This increase in silt deposition raises the bed level and finally affects the life of lakes, dams, tanks, etc. and requires expenditure to be increased for frequent desilting through dredging. Aquatic weeds also affect quality of water. These weeds cause taste and odour problems and also increase biological oxygen demand because of organic loading. They increase the organic matter content of water which may affect the strength of the concrete structures when used as curing and mixing water. It is due to the organic matter that combines with cement to reduce bond strength and may cause large amount of air entrained in concrete.

Aquatic weeds impede the free flow of water which may contribute to increased seepage and

may cause rises in water tables in the adjoining areas. It may lead to water logging. This may also create saline or alkaline conditions in the soil and also give rise to many other land weeds. Submerged and floating weeds propagate at a tremendous rate. The surface floating weeds get interwoven and form dense mats that move downstream. Often these moving mats pack up against bridges and structures creating enormous pressure that sometimes results in serious damage being caused. An example of this sort of damage was observed on Kasur Nala near Taran Taaran in India. Over time if left unchecked, the weed mats become so dense that people and animals can walk on them, although at the risk of injury or drowning.

9 Management of Aquatic Weeds

Considering the losses caused by aquatic weeds, their management is of utmost importance to improve the availability of water from the source to its end users. This does not only improve availability but also the conveyance efficiency. Irrigation and drainage systems provide favourable conditions for the growth of aquatic weeds which interfere with the storage and delivery systems of irrigation water and maintenance of canals, drains, barrages, lakes, ponds, etc. These systems often get choked with the weeds and cause environmental pollution. On low-lying areas, adjoining irrigation and drainage channels, soil salinity and alkalinity problems do arise. Management of aquatic weeds consists of two approaches, namely, preventive and control of existing infestation:

1. Preventive approaches
2. Control

Type of aquatic weeds flora and their intensity influence the damage caused by them. The habitat and the type of aquatic weed flora influence the technique of weed control. In broader sense, weed 'control' means keeping the weeds at a level where they do not cause economic damage. Aquatic weed can be brought under control to manageable limits by various methods. Broadly, these methods can be grouped under four groups:

1. Physical or mechanical methods
2. Biological methods
3. Chemical methods
4. Cultural and physiological methods

There is rarely a situation when weeds can be 'eradicated' but often can be 'prevented' from infesting other areas. Prevention can be useful for a certain weed species or may include a group of aquatic weeds in a given aquatic environment. Once prevention fails, the next step is to eradicate it, that is, treating them in a way that they do not emerge again.

10 Preventive Approaches

Quarantines are legislative tools that may be used to mitigate the effect of weeds. Quarantine is defined as the restriction imposed by duly constituted authorities, whereby the production, movement or existence of plants, plant products, animals, animal products, any other article or material or the normal activity of persons is brought under regulation in order that introduction or spread of a pest may be prevented or restricted. If a pest has already been introduced and established in a small area, a quarantine is necessary so that it may be controlled or eradicated or dissemination stopped in newer areas, thereby reducing the losses that would otherwise occur through damage done by pest. The success of preventive weed management programmes varies with weed species, its biology, means of dissemination and the amount of effort needed to be applied. Preventive weed programmes usually require community action through enactment and enforcement of appropriate laws and regulations.

In India, irrigation canals appear to be a potential source for spreading water hyacinth. Recently preventive weed management approach has been reviewed. When prevention and eradication fail to give desired results under aquatic environment, the only alternative left is to keep aquatic weeds under manageable limits so that water-use efficiency with respect of water

storage in reservoirs and transportation through canals is not reduced. Management of aquatic weeds in water reservoirs, canals, drainage systems, ponds, etc. consists of following systems approach of aquatic weed management, that is, following prevention, eradication and control techniques based on the habitat and type of weed flora present in a given situation. These situations may result in serious reduction in water flow in irrigation and drainage systems which may result in flooding, salinity and alkalinity. Under specific situations, it may adversely influence navigation and operation of turbines in hydroelectric projects.

11 Control Methods

11.1 Physical or Mechanical Control Methods

11.1.1 Mechanical Control of Aquatic Weeds

Mechanical control of aquatic weeds primarily consists of removing the weeds of any group physically from the waterbody. It may also involve any physical power which may directly or indirectly inhibit the growth and development of aquatic weeds. This could be done manually by hand, using hand tools or machine power. It may also consist of altering the environment or creating conditions/situations which may inhibit or do not permit growth and development of weeds. The advantages of mechanical methods include utilisation of available manpower resources, environmental friendly and target specific, yield immediate results, nonselective (under specific situations) and provide fewer chances of permitting ecological shifts in aquatic flora. Mechanical methods often reduce massive nutrient load of eutropic waterbodies, helping indirectly in diminishing the future weed population. Harvested weeds may have various utilities such as feed, manure and energy source, and most importantly, mechanical methods can be exercised in any localised area of waterbodies.

The limitations include limited effectiveness as in some cases aquatic weeds regrow up from their rootstocks, rhizomes. Physical removal especially with machines may help in spreading weeds to new areas, and sometimes, removal of aquatic weeds may deplete waterbodies of their nutrients limiting growth of plantation.

Controlling weeds in an aquatic environment is greatly complicated because of lack of ownership of waterbodies. Most of these are places of public interest. Often frequent approvals are needed from public health department, water surveyor and fish and other wildlife agencies before weed control works may be carried out. In many developing and underdeveloped countries, there is no control on water use. In many Asian countries, a waterbody can be used for a number of purposes including bathing, drinking, stock watering and irrigation.

11.1.2 Manual Cleaning

In areas sparsely infested, weeds can be removed by hand. This could apply to the removal of floating weeds like water hyacinth. Generally, this method is applied to emergent weeds, for example, *Typha* spp., *Phragmites* spp. *and Justicia* spp. (Willow), where men cut the vegetative growth with heavy knives and hooks. In shallow water, the propagules, rhizomes and other underground reproductive organs can be removed.

11.1.3 Cutting

This method consists of physically cutting the biomass over and under the water with the help of heavy knives or mechanical weed cutters. In the case of *Typha*, it has been observed that if plants are cut under the water and remain submerged for more than a week to 10 days, control is possible. This may also hold good for *Phragmites* spp. Also mechanical cutting of water hyacinth and other submersed aquatic weeds like *Chara* spp., filamentous *algae* and *Potamogeton* spp. will give temporary relief from weed infestations. A mechanical weed cutter is used to cut floating and submersed weed at 1–1.5-m depth in water reservoirs. It consists of sharp cutter bar and operates from a boat. The harvested weeds are collected, and water is squeezed from them to hasten dehydration and desiccation.

11.1.4 Chaining

Chaining consists of a heavy iron drag chain attached between two tractors, which is dragged down a densely weed infested ditch or medium canal. The chain tears the rooted weeds and loosens them from the bottom. This method has been found effective where there is dominance of emergent and submersed weeds. The practice of chaining should be followed when new shoots of weed are around 30–50 cm above water level. Dragging the chain up and down the stream may be effective in dislodging most of the weeds. For effective weed control, the practice should be repeated at frequent intervals if found successful. One of the limitations of this method is that ditches need to have uniform width, accessible from both the sides with tractors and free from trees and other such obstructions. The debris thus collected at the end should be removed to avoid reinfestation by plant propagules further downstream.

11.1.5 Water Weed Cutters and Harvesters

In high discharge canals and very large waterbodies, weed cutters/harvesters are used to control rooted submerged weeds:

1. Underwater cutters: These are normally attached to a motorboat. The equipment consists of sharp and strong cutter bars with heavy reciprocating blades, sliding against a fixed blade.
2. Harvesters: Machine that cuts and picks up the weeds from waterbody and conveys these to shore simultaneously.

11.1.6 Dredging

Dredging is one of the techniques by which the weed vegetation along with excess silt is removed from drains and ditches. A dredger is a machine equipped with a forked bucket which can be opened and closed on command. The machine could operate from the ground or from a boat in water. Dredging is done in large waterbodies, canals and drains. It is a common method of cleaning ditches but slow, time-consuming and is a costly operation. Small lakes, water reservoirs, etc. get silted if area surrounding them is under cultivation or surrounded by erodible lands with poor afforestation. When silts get sedimented at bottom, the water retention gets decreased and emergent weeds (*Typha, Scirpus* spp., etc.) establish.

Such a situation demands the use of dredging facilities to remove silt and increase the water capacity of lakes. This also reduces the problem of emergent weeds. Dredging essentially meant for desilting along shores also helps in removal of aquatic weed vegetation.

11.1.7 Mowing

This process consists of cutting the weeds close to the ground with the help of manual or power-operated mowing machines. Mowing is effective on tall growing plants. Repeated mowing not only prevents seed production of emergent weeds but may also starve the underground parts which store carbohydrate reserves and provide energy to vegetative reproductive organs. The best time to mow is when carbohydrate reserves are low. For many species, it is when the active growth phase is over and the time of flowering initiation starts. Repeated mowing hastens carbohydrate depletion and slows death of plants. Generally, this practice effectively controls emergent weeds on canals, water reservoirs, banks, etc. Where gradient in ditches is smooth and not too steep, the underwater cutters can be used to control emergent and submerged weeds. The effect of mowing is short lived. The operation needs to be done frequently to exhaust carbohydrate resources. Therefore, this process does not give any effective control on long-term basis. Pasturing is the economical and effective way in controlling marginal grasses, weeds, etc. A good legume grass mixture if properly managed and grazed will give a lawn-like appearance. A good sod shall also protect banks of canals, drains and dams against erosion. Excessive movement of animals may destroy the banks and make water muddy and may also degrade the quality of the water.

11.1.8 Netting

Scattered floating weeds can be skimmed out of small waterbodies using nets usually made of three mesh coir ropes.

11.1.9 Barriers

Bamboo or inflatable rubber boom fencing is used to restrain the drift of free-floating aquatic weeds. The barriers are made to allow water to pass through them and to sustain the wave and wind action.

11.1.10 Checking Weeds Seeds Through Irrigation Water

Irrigation water often carries the seeds of aquatic weeds such as *Eichhornia crassipes, Pistia stratiotes* and *Salvinia molesta*. It is important to control weeds near and in reservoirs and irrigation canals to prevent them from shedding seeds into the water. Weed seeds can be collected by screens and removed from the source of supply. The screens should be made of woven plastic cloth of less than 1.0-mm mesh supported on rigid metal 1.5-cm screens. Allowing a square metre of screen for each 0.05 m^3s^{-1} of water flow with the fine screen tightly stretched to encourage vibration and self-cleaning as water falls on it. Lining of canals helps in reducing weed vegetation (Yamuna Canal Haryana, India). High velocity of water in flow water system discourages weed growth.

11.1.11 Burning/Fire/Heat Treatment

Aquatic weeds especially emergent bank weeds can also be brought under control with the help of fire. The general thermal death point of most of the weeds is in between 45 and 55°C. Higher-temperature treatment than this result in coagulation of cell protoplasm which inactivates the enzymic process resulting in the death of the plant. The heat treatment required for weed control is provided with the help of fire through flame throwers. Burning may be used to control bank weeds in irrigation canals, ditches, etc. Usually green plants are also given preliminary shearing and, after 2–3 weeks vegetation, may be dry enough to be successfully re-burnt. Burning can be combined with herbicides and mowing to increase its efficiency. Often mowing followed by burning or burning followed by herbicide application on regrowth will help the efficacy of each other treatment.

11.2 Eco-Physiological Alterations

11.2.1 Drying or Water-Level Manipulations

This method is a simple and effective way of controlling submerged weeds. Most of the aquatic weeds respond quickly to changes in water level. Control is achieved by either dehydration of the vegetation or by exposure to low temperatures. In tanks, fish ponds and canals, emptying the water periodically to kill the weeds susceptible to desiccation is practised. To kill submersed weeds in the canals of Bhakra canal system in Haryana (India) and in Chambal command area (India) in Rajasthan, exposure to sun is given by draining the water, and this practice prevents regrowth for nearly 6 months. Cutting of the aerial shots of *Typha* spp. at flowering stage and keeping the stubble submerged under the water for 4 weeks controlled *Typha*. Under such cases, there may be disadvantages in lowering the water level as it may induce production of vegetative propagules or sexual reproduction. Therefore, in such cases, weeds should be removed quickly and the sediments should be dried completely. Planting of trees on the banks of canals may create shade to reduce light intensity hence checking the weed growth. Light intensity can also be checked by adding dyes to the water. This type of control is more effective in static water such as ponds or tanks where dye remains suspended for a longer time. Drying or water-level manipulation is generally practised in flowing water system like irrigation canals and drainage ditches. During the process, the water is removed and the base of the tanks, canals, etc. is made dry by exposing the land to sun and air. This totally changes the eco-environment, which is very adverse to the eco-environment required for growth and development of submerged weeds. Frequent drying and

wetting for several days may control the growth of roots and propagules in the bottom soil. This method is not effective for control of emergent weeds.

11.2.2 Light

Light is an essential component of the photosynthetic process, which is necessary for the growth and development of aquatic plants, especially submersed aquatic weeds. Growth of submerged aquatic plants in small tanks and ponds can be checked by reducing light penetration. Use of fibre glass screen is popular in some countries. Colouring chemicals have also been tried for intercepting solar radiation reaching the water. Ponds that are adequately fertilised develop millions of tiny plants which give the water a cloudy appearance (bloom). If this water is nearly 75 cm deep, submerged aquatic weeds have almost no chance to grow. A light-coloured object should not be visible at around 50 cm below the surface. This practice should be followed where there is little loss of water from the pond. Some object to it as unclean water but that is not the case. The bloom induced by fertiliser application is not considered as bad for health. The proper construction of a tank is very important for controlling pondweeds. Many rooted aquatic plants do not establish in deep water. Therefore, tanks should generally be deeper than 1 m. The slope at the bank should not be more than 2 m, that is, the angle of slope should be steep, that is, around 3:1, and this will help in reducing the area where infestations of *Typha, rushes* and *sedges* could establish. This may be dangerous for access, but flatter separate slopes can be provided at one or two location in the pond for general access.

11.2.3 Breaking of Anchorage

Submerged aquatic weeds can only survive if there is optimum sunlight. In shallow water, optimum light may penetrate to the bed level allowing plants to anchor and take root at the base of the distributary, water course, shallow pond, etc. In case of canals, barrages and tanks with deeper water levels, the light may not reach the bed level. Under such situation, weeds may form anchorage on the inside banks of the irrigation system. In an experiment conducted at Nirwan Branch near Patiala (India), a canal was heavily infested with submerged weeds. Divers cleared the bed of weed. Thereafter, a plough was lowered in the canal along with wooden floats which were connected with a tractor and pulled upstream of canal. However, no weed could be brought out from the bed. It is important to check where the weeds are anchored and growing from so that they can be successfully managed. Alternatively, side walls may be covered with coloured polyethylene to exclude all light penetration and facilitate early decomposition of the plant materials.

11.2.4 Submergence

Typha is one of the most important emergent weed growing all along the unlined canals margins of the waterbodies and shallow submersed areas along canals. Cutting *Typha* close to the ground followed by subsequent submergence or cutting *Typha* under the water provides effective control of this weed.

11.2.5 Competitive Displacement

The approach of replacing harmful vegetation by relatively less harmful and beneficial vegetation needs more research. Planting of Paragrass (*Brachiaria mutica*) in drainage ditches in the Chambal irrigation project eliminated *Typha angustata* after 10–12 months and yielded green fodder. Besides direct competition, growth is also suppressed by some plants by shading effect. For example, the growth of *Azolla* in rice fields effectively controls the growth of other weeds.

11.3 Biological Control of Weeds

Biological management of aquatic weeds is a broad term for the exploitation of living organisms or their products to reduce or prevent the growth and reproduction of weeds. The organisms that are used for biological control are diverse, for example, insects, pathogens, nematodes, parasitic and competing plants. Biological control involves the deliberate use of organisms such as insects or fungi to control weeds.

Biological control is more complex than chemical control because it requires (a) long-term planning, (b) multiple tactics and (c) manipulation of cropping system to interact with the environment and has attempted to work out the total releases made against weeds by biological agents. It was found that after 13 releases of agents for classical control of weeds in the first decade of this century, the number of releases per decade increased nearly exponentially.

11.3.1 Biocontrol Agents

Owing to the increasing awareness about ill effects of herbicides and no control on use of water, lately emphasis is being given to research for nonchemical approaches. Biological control is considered to be one of the most safest approaches. Any plant-feeding organism may be used to control aquatic weeds provided; it does not harm plants of economic value or create undesirable imbalances in the plant community. Some of the natural enemies have been considered for control of submersed, floating and emergent weeds.

11.3.2 Pathogens

Weeds can be controlled by pathogens like fungi, bacteria, virus and virus-like agents. Among the class of pathogens, fungi have been used to a larger extent than bacterial, viral and nematode pathogens. In some cases, it has been possible to isolate, culture, formulate and disseminate fungal propagules as mycoherbicides. Several books and reviews detail the history, development prospects and technical aspects of the use of plant pathogens. Pathogens may have many advantages like (1) most pathogens of plants are fungi, (2) they are destructive, (3) they are widely prevalent, (4) most of them can be easily mass cultured and (5) they can be integrated into organised pest management systems. Most specificity is the fundamental feature. Pathogens with broad host range are unsuitable simply because they may attack the cultivated plants. Formulations of fungi applied as inundative inoculum in a manner similar to that of chemical herbicides have been termed ‘mycoherbicides’. It involves mass culturing, standardisation, formulation and application of fungi inoculum to weeds.

11.3.3 Use of Aquatic Mammals and Rodents

Introduction of manatee (*Trichechus inunguis*) and the rodent *Coypus* (*Myocastor coypus*) both known to feed on aquatic vegetation had earlier been suggested as possible biocontrol agents against aquatic weeds, but the slow reproductive rate of the former and the omnivorous feeding of the latter have discarded their trials.

11.3.4 Use of Fish

Among the several species of herbivorous fishes which feed on aquatic weeds, the more important are *Tilapia melanopleura, T. zilli, T. nilotica* and *Puntius gonionotus*. It is reported that *T. zilli* in the cooling ponds of a power station in Moscow and found it to be a great consumer of weed *Vallisneria*, but this fish cannot survive below 55°F. The Russians who consider fish as more valuable and more permanent agent for weed control than mechanical or chemical are using the grass carp *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*. The former is said to be the more effective species. It feeds on a wide range of aquatic weeds such as *Potamogeton*, *Lemna, Ceratophyllum, Elodea, Hydrocharis*, *Vallisneria* and *Myriophyllum*. The *C. idella* fish has been employed for weed control in China, Hungary and Japan and has shown promise in other regions. It feeds on a range of submerged and floating weeds but prefers plants having soft tissues. Its rate of growth and development varies with the source of food. The white amur displays good performance in high and low temperatures and is not known to reproduce naturally outside its native water.

It is consumed by the herbivorous fish *Ctenopharyngodon idella* (triploid grass carp) under environmental conditions of Northern California Irrigation System. The programme was executed using several initial fish densities

ranging from 0 to 300 kg fish/ha. The data showed that temperature of 12–24°C would require more fish (50 or 250 kg vegetated/ha). It is also concluded that *C. idella* may be an effective and economically feasible option for *P. pectinatus* control in cool water irrigation systems. Complete control of *Hydrilla, Myriophyllum*, *Ceratophyllum, Utricularia* and *Najas* was observed at 1 and 2 fishes (triploid grass carp) per enclosure. When weeds were controlled, there was some damage to water lilies in 2 of the enclosures. In a study testing the preference of grass carp (triploid) on submersed aquatic weeds based on experimental value, triploid grass carp preference was determined as *Potamogeton pectinatus* > *Chara* > *Myriophyllum spicatum*. A filamentous alga *Cladophora* species disappeared the area not surrounded by enclosure after 9 months of introduction of fish.

It is reported grass carp to feed voraciously on *Hydrilla, Azolla, Nechamandra* and *Lemna* spp. In India, ponds choked with *Hydrilla* have been cleared within a month by stocking 300–375 grass carps (weighing 78–173 kg ha^{-1}). White amur is a poor breeder in the warm water; therefore, for weed control purposes, it is bred artificially and released in the water when fingerlings are 100 g each. About 1,500 fingerlings must be released per hectare area of water. Fry and fingerlings of the carp are being distributed to different states in India by Central Inland Fisheries Research Institute, Barrackpore, India.

11.3.5 Use of Snails

Promising results have also been obtained utilising snails *Pomacea canaliculata Lamer*, against the aquatic weed *Anachaares alensa* in Brazil and *Marisa cornuarietis* in Florida. Good results have also been observed against aquatic weeds like *Ceratophyllum demersum, Najas guadalupensis* and *Potamogeton illinoensis* which were controlled completely. *Pistia stratiotes* and *Alternanthera philoxeroides* were partially controlled, while *Eichhornia crassipes* was not completely eaten, but its growth and flowering were greatly retarded by root-pruning action of the snail. The snail *Marisa cornuarietis* feeds on a number of aquatic plants and was considered to have weed control potential. However, its usefulness was limited because of its ability to feed on young rice plants and poor tolerance to water temperature below 10°C. On the other hand, its ability to destroy the breeding sites of the snail vector of *bilharzia* may allow its introduction in non-rice areas. *Pomacea australis*, a South American snail, is also being considered for weed control.

11.3.6 Use of Insects

Water hyacinth (*Eichhornia crassipes*) remains the world's most important aquatic weed. It is spreading at an alarming rate in Africa and Papua New Guinea and is a major problem in the Indian subcontinent and Southeast Asia. Successful biological control can significantly reduce this weed cover in 3–10 years after establishment of an agent and has achieved excellent control in number of countries. The use of curculionid *Neochetina bruchi* for controlling water hyacinth was investigated in Karnataka (India) in 1984. Between February and July, a total of seven releases consisting of 1,700 beetles was made into a 20-ha tank fully infested with water hyacinth. Releases were then confirmed to an area of about 1 ha, and observations on establishment and dispersal of the beetle were made at 2-month interval. By March 1985, upto five adults were present per plant in the release area, and the insects had started dispersing to other parts of the tank. The beetle was present throughout the tank by Sept. 1985. By September 1987, about 90% control of water hyacinth had been achieved, and the remaining plants were stunted with reduced vigour. The curculionid coexists with *N. eichhorniae* which was also released in India from USA for biological control of this weed.

Over 7,500 adults of *Neohydronomus affinis* were released in Florida between April 1987 and July 1988 for biological control of *Pistia stratiotes*. Periodic observations from June 1987 to September 1988 indicated establishment and dispersal of bioagent. At some sites, *N. affinis* infested plants exhibited symptoms typical of plants in other countries where *N. affinis* has been used successfully to control this weed. The

potential of North American aquatic weevil *Euhrychiopsis lecontei* to serve as a bioagent for an exotic weed *Myriophyllum spicatum* which is currently found throughout USA and Southern provinces of Canada was evaluated. This weevil was found on *M. spicatum* in lakes where population of the exotic weed has declined. In both lakes, there was 50% less *M. spicatum* biomass in enclosures with weevils than in enclosures without weevils. The results suggest that the North American insect may be suitable control agent for this introduced aquatic weed.

Analysis of Phytoplankton and Zooplankton: Qualitative and Quantitative

3

1 Introduction

The name plankton comes from the Greek word planktos meaning wanderers (Thurman 1997). There are two types of plankton—phytoplankton and zooplankton. Phytoplankton are plant plankton. Zooplankton are animal plankton. Among the phytoplankton are seaweeds and algae. Phytoplankton are mostly made up of diatoms and dinoflagellates. Diatoms are microscopic, single-celled plants covered by two shells which look glossy. Dinoflagellates are tiny plants with white shells all over them. The shells have a whip-like motion that allows the phytoplankton to move. These are the types of phytoplankton that are found in the sea and they must be protected by all of us.

Phytoplankton lives near the surface of the water body because they need sunlight like all green plants. They also need water and nutrients to live. Phytoplankton use water and CO_2 to grow, but phytoplankton still need other vitamins and minerals, like iron to survive. When the surface of the ocean is cold, the deeper parts of the ocean bring these nutrients to the surface and the plankton live. But, when the surface of the water body is warm, as in El Niño, the water body does not bring as many of these essential nutrients and the phytoplankton die. That causes a major problem because phytoplankton are at the base of the food chain. So, when the population of phytoplankton is reduced almost, the entire food chain is affected. When phytoplankton die, they drop to the bottom of the ocean.

Phytoplankton use CO_2 for survival, which means that the more phytoplankton there are the more CO_2 will be sucked out of the air. When there is less CO_2 in the air, the temperature is going to be lower. When there is a smaller population of phytoplankton, there is more CO_2 in the air. This leads to higher temperatures. Phytoplankton are very important to life and humans should always protect them.

Plankton are any drifting organisms (plants, archaea or bacteria) that inhabit the pelagic zone of water body, seas or bodies of fresh water. That is, plankton are defined by their ecological niche rather than phylogenetic or taxonomic classification. They provide a crucial source of food to larger, more familiar aquatic organisms such as fish and whales.

Though many planktic species are microscopic in size, plankton include organisms covering a wide range of sizes, including large organisms such as jellyfish. Plankton typically flow with ocean currents. While some forms are capable of independent movement and can swim hundreds of metres vertically in a single day, their horizontal position is primarily determined by the surrounding currents. This is in contrast to nekton organisms that can swim against the ambient flow

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and control their position (e.g. squid, fish and marine mammals).

Within the plankton, holoplankton spend their entire life cycle as plankton (e.g. most algae, copepods, salps and some jellyfish). By contrast, meroplankton are only planktic for part of their lives (usually the larval stage) and then graduate to either a nektic or benthic (sea floor) existence. Examples of meroplankton include the larvae of sea urchins, starfish, crustaceans, marine worms and most fish. Plankton abundance and distribution are strongly dependent on factors such as ambient nutrient concentrations, the physical state of the water column and the abundance of other plankton. The widespread use of planktonic in both scientific and popular literature is grammatically incorrect because of the Greek roots of plankton.

2 Trophic Groups

Plankton are primarily divided into broad functional (or trophic level) groups:

Phytoplankton (from Greek *phyton*, or plant), autotrophic, prokaryotic or eukaryotic algae that live near the water surface where there is sufficient light to support photosynthesis. Among the more important groups are the diatoms, cyanobacteria, dinoflagellates and coccolithophores.

Zooplankton (from Greek *zoon*, or animal), small protozoans or metazoans (e.g. crustaceans and other animals) that feed on other plankton and telonemia. Some of the eggs and larvae of larger animals, such as fish, crustaceans and annelids, are included here.

Bacterioplankton, bacteria and archaea play an important role in remineralising organic material down the water column.

This scheme divides the plankton community into broad producer, consumer and recycler groups. However, determining the trophic level of some plankton is not straightforward. For example, although most dinoflagellates are either photosynthetic producers or heterotrophic consumers, many species are mixotrophic depending upon circumstances.

2.1 Size Groups

Plankton are also often described in terms of size (Omori and Ikeda 1992). Usually the following divisions are used:

Group	Size range		
Megaplankton	$>2 \times 10^{-2}$ m	(20+ mm)	Metazoans; e.g. jellyfish; ctenophores; salps and pyrosomes (pelagic Tunicata); Cephalopoda
Macroplankton	$2 \times 10^{-3} \rightarrow 2 \times 10^{-2}$ m	(2–20 mm)	Metazoans; e.g. pteropods; chaetognaths; Euphausiacea (krill); Medusae; ctenophores; salps, doliolids and pyrosomes (pelagic Tunicata); Cephalopoda
Mesoplankton	$2 \times 10^{-4} \rightarrow 2 \times 10^{-3}$ m	(0.2–2 mm)	Metazoans; e.g. copepods; Medusae; Cladocera; Ostracoda; chaetognaths; pteropods; Tunicata; Heteropoda
Microplankton	$2 \times 10^{-5} \rightarrow 2 \times 10^{-4}$ m	(20–200 μm)	Large eukaryotic protists; most phytoplankton; Protozoa (Foraminifera); ciliates; Rotifera; juvenile metazoans—Crustacea (copepod nauplii)
Nanoplankton	$2 \times 10^{-6} \rightarrow 2 \times 10^{-5}$ m	(2–20 μm)	Small eukaryotic protists; small diatoms; small flagellates; Pyrrophyta; Chrysophyta; Chlorophyta; Xanthophyta
Picoplankton	$2 \times 10^{-7} \rightarrow 2 \times 10^{-6}$ m	(0.2–2 μm)	Small eukaryotic protists; bacteria; Chrysophyta
Femtoplankton	$<2 \times 10^{-7}$ m	(<0.2 μm)	Marine viruses

However, some of these terms may be used with very different boundaries, especially on the larger end of the scale. The existence and importance of nano- and even smaller plankton was only discovered during the 1980s, but they are thought to make up the largest proportion of all plankton in number and diversity. The microplankton and smaller groups are microorganisms and operate at low Reynolds numbers, where the viscosity of water is much more important than its mass or inertia (Dusenbery 2009).

2.1.1 Distribution

Plankton inhabit oceans, seas, lakes and ponds. Local abundance varies horizontally, vertically and seasonally. The primary cause of this variability is the availability of light. All plankton ecosystems are driven by the input of solar energy, confining primary production to surface waters and to geographical regions and seasons having abundant light. A secondary variable is nutrient availability. Although large areas of the tropical and subtropical oceans have abundant light, they experience relatively low primary production because they offer limited nutrients such as nitrate, phosphate and silicate. This results from large-scale ocean circulation in water body and water column stratification. In such regions, primary production usually occurs at greater depth, although at a reduced level.

Despite significant macronutrient concentrations, some oceans are unproductive (Martin Martin and Fitzwater 1988). The micronutrient iron is deficient in these regions, and adding it can lead to the formation of blooms of many kinds of phytoplankton (Boyd et al. 2000). Iron primarily reaches the ocean through the deposition of dust on the sea surface. Paradoxically, oceanic areas adjacent to unproductive, arid land thus typically have abundant phytoplankton. While plankton are most abundant in surface waters, they live throughout the water column. At depths where no primary production occurs, zooplankton and bacterioplankton instead consume organic material sinking from more productive surface waters above. This flux of sinking material, so-called marine snow, can be especially high following the termination of spring blooms.

Biogeochemical Significance

Aside from representing the bottom few levels of a food chain that supports commercially important fisheries, plankton ecosystems play a role in the biogeochemical cycles of many important chemical elements, including the ocean's carbon cycle. Primarily by grazing on phytoplankton, zooplankton provide carbon to the planktic food web, either respiring it to provide metabolic energy or upon death as biomass or detritus. Typically more dense than seawater, organic material tends to sink. In open ocean, ecosystems away from the coasts transport carbon from surface to deep waters. This process is known as the biological pump and is one reason that oceans constitute the largest carbon sink on Earth. It might be possible to increase the ocean's uptake of carbon dioxide generated through human activities by increasing plankton production through 'seeding', primarily with the micronutrient iron. However, this technique may not be practical at a large scale. Ocean oxygen depletion and resultant methane production is one potential drawback (Chisholm et al 2001; Aumont and Bopp 2006).

Biomass Variability

The growth of phytoplankton populations is dependent on light levels and nutrient availability, and the chief factor limiting growth varies from region to region in the world's oceans. On a broad scale, growth of phytoplankton in the oligotrophic tropical and subtropical gyres is generally limited by nutrient supply, while light often limits phytoplankton growth in subarctic gyres. Environmental variability at multiple scales influences the nutrient and light available for phytoplankton, and as these organisms form the base of the fresh and marine food web, this variability in phytoplankton growth influences higher trophic levels. For example, at interannual scales phytoplankton levels temporarily plummet during El Nino periods, influencing populations of zooplankton, fishes, sea birds and marine mammals.

The effects of anthropogenic warming on the global population of phytoplankton are an area of active research. Changes in the vertical stratification of the water column, the rate of temperature-dependent biological reactions and

the atmospheric supply of nutrients are expected to have important impacts on future phytoplankton productivity. Additionally, changes in the mortality of phytoplankton due to rates of zooplankton grazing may be significant.

Importance to fish. Zooplankton are the initial prey item for almost all fish larvae as they switch from their yolk sacs to external feeding. Fish rely on the density and distribution of zooplankton to match that of new larvae, which can otherwise starve. Natural factors (e.g. current variations) and man-made factors (e.g. river dams) can strongly affect zooplankton, which can in turn strongly affect larval survival and therefore breeding success.

3 Zooplankton

Individual zooplankton are usually too small to be seen with the naked eye, but some, such as jellyfish, are large. Zooplanktons are of various sizes of organism including small protozoans and large metazoans. It includes holoplanktonic organisms whose complete life cycle lies within the plankton, as well as meroplanktonic organisms that spend part of their lives in the plankton before graduating to either the nekton or a sessile, benthic existence. Although zooplankton are primarily transported by ambient water currents, many have locomotion, used to avoid predators or to increase prey encounter rate. Important protozoan zooplankton groups ecologically include the foraminiferans, radiolarians and dinoflagellates. Metazoan zooplankton which are important include cnidarians such as jellyfish and the Portuguese man-of-war, crustaceans such as copepods and krill, chaetognaths (arrow worms), molluscs such as pteropods and chordates such as salps and juvenile fish. This wide phylogenetic range includes a similarly wide range in feeding behaviour: filter feeding, predation and symbiosis with autotrophic phytoplankton as seen in corals. Zooplankton feed on bacterioplankton, phytoplankton, other zooplankton, detritus and even nektonic organisms. Due to this, zooplankton are primarily found in surface waters where food resources (phytoplankton or other zooplankton) are abundant.

Just as any species can be limited within a geographical region, so is zooplankton. However, species of zooplankton are not dispersed uniformly or randomly within a region of the ocean. Instead 'patches' of zooplankton species (this also applies to phytoplankton) exist throughout the ocean. Though few physical barriers exist above the mesopelagic, specific species of zooplankton are strictly restricted by salinity and temperature gradients, while other species can withstand wide temperature and salinity gradients (Jude et al. 2005). Zooplankton patchiness can also be influenced by biological factors, as well as other physical factors. Biological factors include breeding, predation, concentration of phytoplankton and vertical migration. The physical factor that influences zooplankton distribution the most is mixing of the water column (upwelling and downwelling along the coast and in the open ocean) that affects nutrient availability and, in turn, phytoplankton production (Jude et al 2005).

Through their consumption and processing of phytoplankton and other food sources, zooplankton play a role in aquatic food webs, as a resource for consumers on higher trophic levels (including fish) and as a conduit for packaging the organic material in the biological pump. Since they are typically small, zooplankton can respond rapidly to increases in phytoplankton abundance, for instance, during the spring bloom. Zooplankton can also act as a disease reservoir. They have been found to house the bacterium *Vibrio cholerae*, which causes cholera, by allowing the cholera vibrios to attach to their chitinous exoskeletons. This symbiotic relationship enhances the bacterium's ability to survive in an aquatic environment, as the exoskeleton provides the bacterium with carbon and nitrogen.

4 Collection and Preservation of Plankton

Zoo- and phytoplankton may be collected by filtering a known amount of water through a

plankton net made up of bolting silk for zooplankton and (No. 25, i.e. mesh size 55 μm) for phytoplankton. Surface water may be collected with the help of plastic bucket of known volume but, for subsurface water, water samples are considered to be better device.

4.1 Plankton Net

When over all phytoplankton and zooplankton composition and population is to be analysed, various types of plankton nets are used, but a simple conical net is used for many years. It is conical in shape and made up of standard silk bolting cloth number 22 having 75 meshes/linear centimetre. The diameter of top metal ring is 36 cm, at the bottom of which graduated 10-ml vial is tied firmly.

The net is completely immersed below the water surface to desired depth. Net is then hauled against the water current to a desired distance. (1) The contents of the specimen tube are transferred to another tube without the loss of samples. The hauling is repeated five times to collect replicates. The samples are well shaken and immediately 5.0 ml of sample is taken out in another tube for plankton counting. One portion is preserved in 4% formaline for zooplanktons and in Lugol's iodine solution for phytoplankton identification. The volume of water filtered through the net is calculated as follows:

$$V = r^2 l$$

V = volume of filtered water
r = radius of plankton ring
l = column of water filtered

4.1.1 Water Sampler

Water sampler is used for collection of vertical column. Sampler has a wide mouth, rubber stopper bottle (2–5 l capacity), covered with an iron sheet or zinc sheet container. The space between the bottle and the container is filled with sawdust. The rubber stopper has a thermometer, an inlet and an outlet tube. Outlet tube is attached to a long plastic tube with a stopper clip at its free end. The container is then placed in a strong netted bag provided at its base with a weight. The nylon bag is tied to a rope marked in metres. As these samples collect whole water samples, all sized classes of phytoplankton are collected.

Sampling Procedure

The sampler is slowly dipped in water body to a desired depth. The stopper clip is pressed by the operator, and the water rushes inside the bottle to fill the air space. The clip stopper is closed and sampler is lifted up. The temperature of water at particular depth is recorded. The rubber stopper is then removed and filtered the water for plankton. Since the water sampler collects whole water samples, all size planktons are collected. Different sizes of plankton may be separated subsequently from whole water samples through netting by the appropriate mesh size. Appropriate mesh sizes are selected for concentrating the various size categories of plankton typical of aquatic system under study. For collection of micro-zooplankton such as protozoans, rotifers and immature crustaceans, 20- to 200-μm net is used. The larger and mature crustaceans may be concentrated by passing through a 20-μm mesh net (APHA 2005).

Plankton Counting

Plankton should be enumerated using a counting cell chamber that limits the volume and area for ready calculation. Plankton can be counted by the following two methods: The 10 ml sample is collected in graduated tube and is subjected to centrifugation with the help of centrifuge at 3,000 rpm for 20 min. Supernatant fluid is decanted and concentrate is thoroughly mixed and used for enumeration.

5 Simplified Method

Shake the sample thoroughly. Out of unfixed 5.0-ml sample, take out 1.0-ml sample in calibrated pipette. The sample is then placed drop by drop over a clear glass slide and cover it with cover glass. Count the number of the total

organisms (zoo- and phytoplankton separately) in each drop in 1.0-ml sample. Sum up the number of organisms of all the drops. The number will represent the total organisms in 1.0 ml of sample (Edmondson 1974).

Formula used for calculation of phytoplankton as units/l is

$$\frac{\text{Plankton density unit}}{\text{l}} = \frac{n \times c \times 1{,}000}{V}$$

where

n = no. of plankton counted in 1.0-ml concentrate

c = total volume of concentrate in ml

V = total volume of water filtered through net.

The identification of the plankton will be made by standard keys and books by Palmer (1980) and APHA (2005).

5.1 Microtransect Method

Take out 0.1 ml of the concentrated plankton sample (after thorough mixing) over a clean slide with the help of dropper. Cover with 22 mm × 22 mm cover slip. Count the organisms in five strips each having six different places. Each visible area will represent one microtransect. Then the area or the transect and width of high power of microscopic field are determined. The counting is done immediately to avoid drying of the drop. Repeat the process with at least five drops (Lackey 1938). This process is repeated five times. Identification of the phytoplankton is done up to genera level with the help of standard keys and books by Palmer (1980) and APHA (2005).

$$\text{Plankton density}\left(\frac{\text{number}}{\text{l}}\right) = \frac{a^2 \times v^1}{V}$$

where

Total number of organisms per drop (0.1 ml) a^1

Number of planktons per ml $a^2 = a^1 \times 10$

a^2 = number of plankton per ml

v^1 = volume of concentrate collected in specimen tube

V = volume of total water filtered through plankton net.

5.1.1 Counting by Sedgewick Rafter Cell (S-R)

The Sedgewick cell is a device commonly used for plankton counting (approximately 50 mm long × 20 mm wide × 1 mm deep). The total area of the bottom is approximately 1.0 ml.

Before filling the S-R cell with sample, place the cover slip diagonally across the cell, formation of air bubbles in cell corner. Before counting let the S-R. cell, stand at least 15 min to settle plankton. Count the plankton on the bottom of the S-R. cell with the help of keys and books under inverted microscope at 100×–200× magnification for phytoplankton. Using compound microscope and magnification of 100×, enumerate zooplankton (protozoa, rotifer and nauplii). A strip is about 50 mm long, 1 mm deep and width 0.5 mm (Palmer 1980; APHA 2005).

$$\frac{\text{No}}{\text{ml}} = \frac{C \times 1{,}000\,\text{mm}^2}{L \times D \times W \times S}$$

where

C = number of organism counted

L = length of each strip (S-R. cell length) mm

D = depth of strip (Whipple grid image width) mm

S = number of strips counted

Counting by Haemocytometer

Another readily available chamber is the standard medical haemocytometer used for enumerating blood cells. It has ruled grid machined into a counting plate in fitted with a ground-glass cover slip. The grid is divided into 1.0 mm^2 division; the chamber is 0.1 mm deep. The sample is then introduced below the cover slip by the pipette and viewed under 450 × magnification. Keep the slide undisturbed for 1–2 min to settle the suspension. Cells are

counted in four large squares chosen random. The large square measures 1 mm long with slide. Therefore, the entire grid is 16 mm^2. haemocytometers 0.2 mm deep. Therefore, value of the sample that covers the grid of nine large squares is 0.009 ml (APHA 2005).

6 Enumeration of Zooplankton (Small)

An open counting chamber 80 by 50 and 2 mm deep, with circular grooves or partitions of 1–5 ml capacity for small zooplankton (protozoans, rotifers and nauplii) and 5–10 ml for larger mature microcrustaceans, is used for counting. A mild detergent is placed on the chamber before counting to reduce movements of organisms. Count the organisms using compound microscope and a magnification of 100 ×. A Sedgewick Rafter cell is not suitable because of size. Smaller zooplankton as number per litre and larger forms as number per cubic metre should be reported.

$$\frac{\text{No.}}{\text{m}^3} = \frac{C \times V^1}{V^{11} \times V^{111}}$$

where
C = number of organism counted
V^1 = value of the concentrated sample
V^{11} = volume counted ml
V^{111} = volume of the grab sample m^3.

To obtain organism per litre, it is divided by 1,000.

6.1 Diversity Indices

Species diversity is a statistical abstraction with two components reflecting the number of species and distribution of individual of all species at a particular site. The two types of diversity indices are computed. Following diversity indices are commonly used.

6.1.1 Odum's Species per Thousand Individuals

Odum et al. (1960) proposed that the species per thousand individuals can be used as an index of diversity which is basically an evenness and is a very reliable index. It is calculated as

$$\text{Odum's index} = \frac{\text{No. of total species in the sample}}{\text{No. of individuals of all species.}} \times 1,000.$$

Shannon's Species Diversity Index (H')

It is an expression of correlation with the pollution status of the ecosystem. This is based on Shannon's information theory. It has been calculated as

$$H\prime = \sum_{1-1}^{s} \left(\frac{\text{ni}}{N}\right) \log_2 \left(\frac{\text{ni}}{N}\right)$$

where
H'_3 = Shannon's index
S = no. of species in sample (genus here)
ni = no. of individuals each species (genus here)
N = total no. of individual in sample (i.e. N = ni)

$$\log_2 = 1.433 \frac{\text{ni}}{N}$$

1.433 = amplification factor.

The diversity can be calculated using any one type of the organisms like diatoms all algae, between macro invertebrates, periphyton or zooplanktons. A relationship of species diversity and pollution status of sampling sites is as follows:
>3 Clean water
1–3 Moderately polluted
<Heavily polluted

A slightly different theory was proposed by a scientist in different scale of pollution in terms of species diversity index, which is modified one

and states a negative correlation between Shannon and Weaver index and pollution:
3.0–4.5 Slight
2.0–3.0 Light
1.0–2.0 Moderate
0.0–1.0 Heavy

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Application of Diversity Index in Measurement of Species Diversity

4

1 Introduction

A diversity index is a statistic which is intended to measure the local members of a set consisting of various types of objects. Diversity indices can be used in many fields of study to assess the diversity of any population in which each member belongs to a unique group, type or species. For instance, it is used in ecology to measure biodiversity in an ecosystem, in demography to measure the distribution of population of various demographic groups, in economics to measure the distribution over sectors of economic activity in a region and in information science to describe the complexity of a set of information. The most commonly used diversity indices are simple transformations of the effective number of types, but each diversity index can also be interpreted in its own right as a measure corresponding to some real phenomenon but a different one for each diversity index (Hill 1973; Jost 2006; Tuomisto 2010a, b).

A series of diversity indices are:

1. Species richness
2. Species evenness
3. Concentration ratio
4. Simpson's diversity index
5. Shannon's diversity index
6. Berger–Parker's index
7. Indices that measure lack of diversity
8. Renyi entropy

2 Species Richness

The species richness is simply the number of species present in an ecosystem. This index makes no use of relative abundances. In practice, measuring the total species richness in an ecosystem is impossible, except in very depauperate systems. The observed number of species in the system is a biased estimator of the true species richness in the system, and the observed species number increases nonlinearly with sampling effort. Thus, S, if indicating the observed species richness in an ecosystem, is usually referred to as species density.

3 Species Evenness

The species evenness is the relative abundance or proportion of individuals among the species.

4 Concentration Ratio

Concentration ratio is a crude indicator of the extent to which a few groups such as species, demographic groups or companies dominate an environment, the total share taken by the top n species or firms. However, by itself, the concentration ratio does not indicate how much that share is divided between those top n firms or species.

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5 Simpson's Diversity Index

If p_i is the fraction of all organisms which belong to the ith species, then Simpson's diversity index is most commonly defined as the statistic

$$D = \sum_{i=1}^{S} p_i^2.$$

This quantity was introduced by Edward Hugh Simpson in 1949. The Herfindahl index in competition economics is essentially the same.

If n_i is the number of individuals of species i which are counted, and N is the total number of all individuals counted, then

$$\frac{\sum_{i=1}^{S} n_i(n_i - 1)}{N(N-1)}$$

is an estimator for Simpson's index for sampling without replacement.

It is noted that $0 \leq D \leq 1$, with values near zero corresponding to highly diverse or heterogeneous ecosystems and values near one corresponding to more homogeneous ecosystems. Biologists who find this confusing sometimes use $1/D$ instead; confusingly, this reciprocal quantity is also called Simpson's index. Another response is to redefine Simpson's index as

$$\tilde{D} = 1 - D = 1 - \sum_{i=1}^{S} p_i^2,$$

This quantity is called by statisticians the index of diversity.

In sociology, psychology and management studies, the index is often known as Blau's index, as it was introduced into the literature by the sociologist Peter Blau.

In economics, essentially the same quantity is called the Hirschman–Herfindahl index (HHI), defined as the sum of the squares of the shares in the population across groups (with E as the group size, i.e. the number of employees or the number of specimen):

$$D = \sum_{i=1} \left(\frac{E_i}{E}\right)^2.$$

Note that a HHI is also used within sectors to measure competition.

The index of diversity (also referred to as the *index of variability*) is a commonly used measure, in demographic research, to determine the variation in categorical data.

Gibbs and Martin defined Simpson's diversity index for use in sociology as

$$D = 1 - \sum_{i=1}^{N} p_i^2$$

where

p = proportion of individuals or objects in a category

N = number of categories.

A perfectly homogeneous population would have a diversity index score of 0. A perfectly heterogeneous population would have a diversity index score of 1 (assuming infinite categories with equal representation in each category). As the number of categories increases, the maximum value of the diversity index score also increases (e.g. 4 categories at 25% = 0.75 and 5 categories with 20% = 0.8).

An example of the use of the index of diversity would be a measure of racial diversity in a city. Thus, if Sunflower City was 85% white and 15% black, the index of diversity would be 0.255.

The interpretation of the diversity index score would be that the population of Sunflower City is not very heterogeneous but is also not homogeneous.

6 Shannon's Diversity Index

Shannon's diversity index is simply the ecologist's name for the communication entropy introduced by Claude Shannon:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i is the fraction of individuals belonging to the ith species. This is by far the most widely used diversity index. The intuitive significance of this index can be described as follows. Suppose we devise binary code words for each species in our ecosystem, with short code words used for the most abundant species, and longer code words for rare species. As one walks around and observes individual organisms, one calls out the corresponding code word. This gives a binary sequence. If we have used an efficient code, we will be able to save some breath by calling out a shorter sequence than would otherwise be the case. If so, the average code word length one calls out as one wanders around will be close to the Shannon diversity index.

It is possible to write down estimators which attempt to correct for bias in finite sample sizes, but this would be misleading since communication entropy does not really fit expectations based upon parametric statistics. Differences arising from using two different estimators are likely to be overwhelmed by errors arising from other sources. Current best practice tends to use bootstrapping procedures to estimate communication entropy.

Shannon himself showed that his communication entropy enjoys some powerful formal properties, and furthermore, it is the unique quantity which does so. These observations are the foundation of its interpretation as a measure of statistical diversity (or 'surprise', in the arena of communications). The applications of this quantity go far beyond the one discussed here; see the textbook cited below for an elementary survey of the extraordinary richness of modern information theory.

7 Berger–Parker Index

The Berger–Parker diversity index is simply

$$\max_1 \leq i \leq S \, p_i.$$

This is an example of an index which uses only partial information about the relative abundances of the various species in its definition.

8 Indices That Measure Lack of Diversity

There are many income inequality indices such as Gini index, Theil index, Hoover index, Robin Hood index, Atkinson index, Suits index and generalised entropy index that increase as diversity decreases, so that they can be used to measure diversity if it is understood that a smaller value represents higher diversity. The values of these indices can be viewed as representing a lack of diversity (isolation or segregation), redundancy, inequality, non-randomness or compressibility in the data.

The Theil index is the maximum possible diversity $\log(N)$ minus Shannon's diversity index. It is the maximum possible entropy of the data minus the observed entropy. The Theil index is called redundancy in information theory.

9 Renyi Entropy

The species richness, the Shannon index, Simpson's index and the Berger–Parker index can all be identified as particular examples of quantities bearing a simple relation to the Renyi entropy

$$H_\alpha = \frac{1}{1-\alpha} \log \sum_{i=1}^{S} p_i^\alpha$$

for α approaching 0, 1, 2, ∞, respectively.

Unfortunately, the powerful formal properties of communication entropy do not generalise to Renyi's entropy, which largely explains the much greater power and popularity of Shannon's index with respect to its competitors.

9.1 Importance

Diversity indices provide important information about rarity and commonness of species in a community. The ability to quantify diversity in this way is an important tool for biologists trying to understand community structure.

Method to measure diversity.

9.1.1 Variables

H	Shannon's diversity index
S	Total number of species in the community (richness)
p_i	Proportion of S made up of the ith species
E_H	Equitability (evenness)

Methods: The Shannon diversity index (H) is another index that is commonly used to characterise species diversity in a community. Like Simpson's index, Shannon's index accounts for both abundance and evenness of the species present. The proportion of species i relative to the total number of species (p_i) is calculated and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species and multiplied by -1:

$$H = -\sum_{i=1}^{S} p_i \ln p_i$$

Shannon's equitability (E_H) can be calculated by dividing H by $H_{\max}$ (here, $H_{\max} = \ln S$). Equitability assumes a value between 0 and 1 with 1 being complete evenness.

$$E_H = \frac{H}{H_{\max}} = \frac{H}{\ln S}$$

For the first case, E_H is always equal to one (complete evenness, or equitability), but H increases dramatically as the number of species increases, as one would expect. For the second case, in which one species makes up 90% of the community, the picture is a little different. Here, we can see that although H does increase with increasing numbers of species, it does so much more slowly than in the first case. Additionally, E_H decreases as species number increases (since one species always makes up 90% of the community in the second case of this hypothetical example, the remaining species make up some fraction of 10% of the community; as species number increases, this fraction becomes smaller and evenness decreases). H and E_H clearly give more information about these communities than would species number (richness) alone.

Different levels of disturbance have different effects on diversity. If our goal is to preserve biodiversity in a given area, then there is a need to be able to understand how diversity is impacted by different management strategies. Because diversity indices provide more information than simply the number of species present (i.e. they account for some species being rare and others being common), they serve as valuable tools that enable biologists to quantify diversity in a community and describe its numerical structure.

10 Diversity Indices

This menu can be used to calculate measures of diversity and bootstrap confidence intervals. A diversity index is a measure of species diversity within a community that consists of co-occurring populations of several (two or more) different species. There are many different diversity indices that combine species richness and evenness, the two elements of diversity, in different ways. Among these indices are the log-series alpha and log-normal lambda which are estimated by fitting an underlying species abundance model and the Q statistic which is derived from cumulative ranked frequencies. Other available indices include the Margalef and Simpson's 1-D which emphasise the richness component of diversity. The indices that highlight the evenness component of diversity include Simpson's 1-D, McIntosh D and E, Shannon–Weiner H' and J', Brillouin diversity and evenness index, Berger–Parker and Smith–Wilson evenness measure. Confidence intervals for the measures can be estimated by bootstrapping. For multiple samples, the overall values of the diversity indices are calculated; an option is available to perform jackknifing to produce less bias estimates with a confidence interval.

11 Ecodiversity

Ecodiversity can be used to calculate several different measures of diversity. Among these indices are the log-series α and log-normal λ

which are estimated by fitting an underlying species abundance model, and the Q statistic which is derived from cumulative ranked frequencies. Other available indices include the Margalef and Simpson's 1-D which emphasise the richness component of diversity. The indices that highlight the evenness component of diversity include Simpson's 1-D, McIntosh D and E, Shannon–Weiner H' and J', Brillouin diversity and evenness index, Berger–Parker and Smith–Wilson evenness measure. Confidence intervals for the measures can be estimated by bootstrapping. For multiple samples, ecodiversity calculates the overall values of the diversity indices and provides an option to perform jackknifing to produce less bias estimates with a confidence interval.

The numbers of individuals per species are specified using the Individual parameters. The SPECIES parameter specifies a variate containing the number of species for the associated number of individuals denoted in the corresponding element of Individuals. SPECIES can be omitted if each of the values in INDIVIDUALS corresponds to one species. The GROUPS option can be used to calculate measures of diversity for different samples. The SAVE parameter allows the diversity indices to be saved in a variate or in a pointer to a set of variates for each group.

The PRINT option controls printed output, with settings:

Index: the index of diversity or evenness

Estimate: bootstrap or jackknife estimate with confidence limits for the statistic

The BMETHOD option can be used to select either the bootstrap or jackknife (for multiple samples) method to produce an estimate of the diversity measure with an associated confidence interval. To produce a bootstrap or jackknife estimate for multiple samples, each sample must contain the same number of values where each element corresponds to the same species within each sample. For the calculation of the bootstrap confidence intervals of the diversity measures, the NBOOT option specifies how many bootstrap samples to take (default 100). The probability level for the confidence interval can be set by the CIPROBABILITY option: by default 0.95. The SEED option specifies the seed to use in the random number generator used to construct the bootstrap samples. The default value of zero continues an existing sequence of random numbers or, if the generator has not yet been used in this run of GenStat, it initializes the generator automatically.

Options: PRINT, INDEX, GROUPS, BMETHOD, NBOOT, SEED, CIPROBABILITY

Parameters: INDIVIDUALS, SPECIES, SAVE

12 Data Format

Specifies formatting of the data. First single variate is selected to supply the individuals in a single variate. Alternatively, multiple variates are selected, if the data are to be supplied in multiple samples of individuals.

13 Individuals

A variate containing the numbers of individuals per species.

13.1 Species

A variate containing the number of species for the associated number of individuals in the corresponding element of Individuals. This field can be left empty if each of the values in Individuals corresponds to one species.

13.1.1 Groups

A factor specifying the groups for the different samples.

Samples

For multiple samples, this allows you to specify one or more variates containing the individuals for different samples. Multiple selections can be

transferred from the Available data list by clicking the button.

Indices
It controls the type of index that is to be calculated. One can select one or more of the indices.

Available Data
This lists data structures appropriate to the current input field. The contents will change as one moves from one field to the next. Double-click on a name to copy it to the current input field; alternatively, one can type the name directly into the input field.

14 Calculating Community Similarity and Diversity Indices

Biological systems are organised on many different levels: molecules, cells, organisms, populations, communities and ecosystems. Species diversity is a characteristic unique to the community level of biological organisation. Higher species diversity is generally thought to indicate a more complex and healthier community because a greater variety of species allows for more species interactions, hence greater system stability, and indicates good environmental conditions. A variety of diversity indices can be calculated to compare ecological communities. In addition, pairs of communities can be compared using community similarity indices.

Species diversity has two parts. Richness refers to the number of species found in a community, and evenness refers to the relative abundance of each species. A community is said to have high species diversity if many nearly equally abundant species are present. If a community has only a few species or if only a few species are very abundant, then species diversity is low. Consider a community with 100 individuals distributed among 10 species. It should make sense that if there are 10 individuals in each of the 10 species in the community, it is more diverse than if there are 91 individuals in one species and one individual in each of the other nine species.

The data obtained from the fish collected in Northrup Creek into a spreadsheet, create formulas and calculate indices to compare the fish communities above and below the WTP and reach conclusions about the similarity and diversities of the two communities.

Exercise 1: Calculating the Proportional Index of Community Similarity.
A good way to compare communities in different places or at different times is to examine community similarity. You will use a simple measure, called Proportional Similarity, to compare fish communities in Northrup Creek. Table 4.1 gives an example of this method.

PS (Percent Similarity) = (lowest percent value of a species between communities), in this case:

$$\begin{aligned} \mathrm{PS} &= 0\,\% + 6\,\% + 13\,\% + 6\,\% + 0\,\% \\ &= 25\,\% \end{aligned} \tag{4.1}$$

1. PS is calculated for the fish communities above/below the WTP discharge to Northrup Creek.

Exercise 2: Simpson's Index of Diversity.
Simpson's index calculates the probability that two organisms sampled from a community of will belong to different species (the more even the abundance of individuals across species, the higher the probability that the two individuals sampled will belong to different species). Simpson's index values range from 0 to 1, with 1 representing perfect evenness (all species present in equal numbers). The formula for Simpson's index is

Table 4.1 The percent of fish sampled in each species in community 1 and community 2 (e.g. 50 = # of species 1 captured in community 1; 93 = total # of fish captured in community 1)

Species	Community 1 (%)	Community 2 (%)
1	50/93 = 54	0/112 = 0
2	25/93 = 27	7/112 = 6
3	12/93 = 13	15/112 = 13
4	6/93 = 6	30/112 = 27
5	0/93 = 0	60/112 = 54
Total	93 fish sampled	112 fish sampled

$$D_S = 1 - \mathrm{Sum}_1^i[n_i * (n_i - 1)]/[N * (N - 1)] \quad (4.2)$$

where = add all n_i*(n_i−1) values together, n_i = the number of individuals in the ith species collected and N = the total number of organisms in the sample. For example, suppose you collected 3 species with 40, 25 and 15 individuals, respectively.

$$\begin{aligned} D_S &= 1 - \frac{40(39) + 25(24) + 15(14)}{80(79)} \\ &= 1 - \frac{2370}{6320} \\ &= 1 - 0.375 \\ &= 0.625 \end{aligned}$$

2. Calculate SI for the fish communities above/below the WTP discharge to Northrup Creek.

Exercise 3: Determining Statistically Significant Differences in Simpson's Diversity.
Inevitably, the D_s values calculated for each community sample will be different. How can one tell if the communities have significantly different D_s values or not? To answer this question requires using statistics, a branch of mathematics that allows you to determine with a known degree of reliability how likely it is that two or more groups are the same or not. To make this kind of comparison, you need to calculate the variability in the data you collected (the more variable the data, the greater the difference in the diversity values has to be to show a significant difference between any two D_s values). The formula to calculate the variance (s^2) of Simpson's index is

$$s^2 = 4\left[p_i^3 - \left(p_i^2\right)^2\right]/N, \quad (4.3)$$

where p_i is the proportion of the number of organisms in the ith species (n_i) to the total number of organisms in the sample (N). Therefore, $p_i = n_i/N$ (these are the same values you used to calculate Simpson's diversity). Using the data from the example from Eq. 4.2, you can calculate s^2.

$$\begin{aligned} s^2 &= 4\left\{\left[(40/80)^3 + (25/80)^3 + (15/80)^3\right]\right. \\ &\quad \left. -\left[(40/80)^2 + (25/80)^2 + (15/80)^2\right]^2\right\}/80 \\ &= 4\{[0.125 + 0.031 + 0.007] \\ &\quad -[0.250 + 0.098 + 0.035]^2\}/\ 80 \\ &= 4\left\{[0.163] - [0.383]^2\right\}/\ 80 \\ &= 4(0.160)/\ 80 \\ &= 0.0008 \end{aligned}$$

You will use a t test to determine whether or not Simpson's diversity values are different for the fish communities above and below the WTP.

$$t = [\text{avg. } 1 - \text{avg. } 2]/\left[\mathrm{sqrt}\left(s_1^2 + s_2^2\right)\right] \quad (4.4)$$

For example, suppose that diversities in two hypothetical communities are 0.8 and 0.3, respectively, and that the variances of the two diversity estimates are 0.03 $\left(s_1^2\right)$ and 0.01 $\left(s_2^2\right)$, respectively.

$$\begin{aligned} t &= 0.8 - 0.3 \\ &\quad \mathrm{sqrt}(0.03 + 0.01) \\ &= 0.5/0.2 \\ &= 2.5 \end{aligned}$$

For this example, if the degrees of freedom (# taxa in community 1 + # taxa in community 2–2 = n_1 + n_2−2) are 8, the t-table value is 2.306. For any calculated value of 't' greater than the number found in the table, the difference in diversity between the two communities is

considered to be significant. For any calculated value of 't' less than the number one finds in the table, the difference in diversity between the two communities is not considered to be significant. For these calculations, statistical significance means there is 1 chance in 20 (5%) that the data shows a difference in community diversity values when in reality there is no difference.

A spreadsheet is used to calculate s^2 for the fish communities above and below the WTP discharge to Northrup Creek and do a t test.

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Challenges of Fish Diversity in Polluted Water

5

1 Introduction

The term 'fish' most precisely describes any non-tetrapod craniate (i.e. an animal with a skull and in most cases a backbone) that has gills throughout life and whose limbs, if any, are in the shape of fins (Nelson 2006a, b). Unlike groupings such as birds or mammals, fishes are not a single clad but a paraphyletic collection of taxa, including hagfishes, lampreys, sharks and rays, ray-finned fish, coelacanths and lungfish (Helfman et al. 2009). Indeed, lungfishes and coelacanths are closer relatives of tetrapods (such as mammals, birds, amphibians) than of other fish such as ray-finned fish or sharks, so the last common ancestor of all fish is also an ancestor to tetrapods. As paraphyletic groups are no longer recognised in modern systematic biology, the use of the term 'fish' as a biological group must be avoided. Many types of aquatic animals commonly referred to as 'fish' are not fish; examples include shellfish, cuttlefish, starfish, crayfish and jellyfish. In earlier times, even biologists did not make a distinction—sixteenth-century natural historians classified also seals, whales, amphibians, crocodiles, even hippopotamuses, as well as a host of aquatic invertebrates, as fish. However, all mammals, including cetaceans like whales and dolphins, are not fish. In some contexts, especially in aquaculture, the true fishes are referred to as finfish (or fin fish) to distinguish them from these other animals.

A typical fish is ectothermic; has a streamlined body for rapid swimming; extracts oxygen from water using gills or uses an accessory breathing organ to breathe atmospheric oxygen; has two sets of paired fins, usually one or two (rarely three) dorsal fins, an anal fin and a tail fin; has jaws; has skin that is usually covered with scales; and lays eggs. Tuna, swordfish and some species of sharks show some warm-blooded adaptations—they can heat their bodies significantly above ambient water temperature. Streamlining and swimming performance varies from fish such as tuna, salmon and jacks that can cover 10–20 body-lengths per second to species such as eels and rays that swim no more than 0.5 body-lengths per second. Many groups of freshwater fish extract oxygen from the air as well as from the water using a variety of different structures. Lungfishes have paired lungs similar to those of tetrapods, and gouramis have a structure called the labyrinth organ that performs a similar function, while many catfish, such as *Corydoras*, extract oxygen via the intestine or stomach. Body shape and the arrangement of the fins are highly variable, covering such seemingly un-fishlike forms as seahorses, pufferfish, anglerfish and gulpers. Similarly, the surface of the skin may be naked or covered with scales of a variety of different types usually defined as placoid (typical of sharks and rays), cosmoid (fossil lungfish and coelacanths), ganoid, cycloid and ctenoid (last two are found on most bony fish).

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There are even fish that live mostly on land. Mudskippers feed and interact with one another on mudflats and go underwater to hide in their burrows. The catfish *Phreatobius cisternarum* lives in underground, phreatic habitats, and its relative lives in waterlogged leaf litter. Fish range in size from the huge 16-m whale shark to the tiny 8-mm stout infant fish.

Fish species diversity is roughly divided equally between marine (oceanic) and freshwater ecosystems. Coral reefs in the Indo-Pacific constitute the centre of diversity for marine fishes, whereas continental freshwater fishes are most diverse in large river basins of tropical rainforests, especially the Amazon, Congo and Mekong basins. More than 5,600 fish species inhabit Neotropical freshwaters alone, such that Neotropical fishes represent about 10% of all vertebrate species on the Earth.

2 By Species

Fishes	Jawless	Lampreys
		Hagfish
	Cartilaginous	Sharks
		Rays
		Chimaera
Bony fishes	Lobe finned	Lungfish
		Coelacanths
	Ray finned	Chondrosteans
		Holosteans
		Teleosts

Basic taxonomy of fishes

Fish systematics is the formal description and organisation of fish taxa into systems. It is complex and still evolving (Nelson 2006a, b). Controversies over 'arcane, but important, details of classification are still quietly raging'. The term 'fish' describes any non-tetrapod chordate (i.e. an animal with a backbone) that has gills throughout life and has limbs, if any, in the shape of fins (Nelson 2006a, b). Unlike groupings such as birds or mammals, fishes are not a single clad but a paraphyletic collection of taxa, including jawless, cartilaginous and skeletal types (Helfman et al. 2009).

3 Jawless Fish

Jawless fishes are the most primitive fish. There is current debate over whether these are really fish at all. They have no jaw, no scales, no paired fins and no bony skeleton. Their skin is smooth and soft to the touch, and they are very flexible. Instead of a jaw, they possess an oral sucker. They use this to fasten on to other fish and then use their rasp-like teeth to grind through their host's skin into the viscera. Jawless fishes inhabit both fresh and salt water environments. Some are anadromous, moving between both fresh and salt water habitats. Extant jawless fishes are either lamprey or hagfish. Juvenile lamprey feed by sucking up mud containing microorganisms and organic debris. The lamprey has well-developed eyes, while the hagfish has only primitive eyespots. The hagfish coats itself and carcasses, it finds with noxious slime to deter predators, and periodically ties itself into a knot to scrape the slime off. It is the only invertebrate fish and the only animal which has a skull but no vertebral column (Campbell and Reece 2005). It has four hearts, two brains and a paddle-like tail (Aird 2007).

4 Cartilaginous Fish

Cartilaginous fishes have a cartilaginous skeleton. However, their ancestors were bony animals and were the first fish to develop paired fins. Cartilaginous fishes do not have swim bladders. Their skin is covered with placoid scales (dermal denticles) that are as rough as sandpaper. Because cartilaginous fishes do not have bone marrow, the spleen and special tissue around the gonads produce red blood cells. Their tails can be asymmetric, with the upper lobe longer than the lower lobe. Some cartilaginous fishes possess an organ called Leydig's organ which also produces red blood cells. There are over

980 species of cartilaginous fish. They include sharks, rays and chimaera.

5 Bony Fish

Bony fishes include the lobe-finned fish and the ray-finned fish. The lobe-finned fish is the class of fleshy-finned fishes, consisting of lungfish and coelacanths. They are bony fish with fleshy, lobed paired fins, which are joined to the body by a single bone (Clack 2002). These fins evolved into the legs of the first tetrapod land vertebrates, amphibians. Ray-finned fishes are so-called because they possess lepidotrichia or 'fin rays', their fins being webs of skin supported by bony or horny spines ('rays').

There are three types of ray-finned fishes: the chondrosteans, holosteans and teleosts. The chondrosteans and holosteans are primitive fishes sharing a mixture of characteristics of teleosts and sharks. In comparison with the other chondrosteans, the holosteans are closer to the teleosts and further from sharks.

6 Teleosts

Teleosts are the most advanced or 'modern' fishes. They are overwhelmingly the dominant class of fishes (or for that matter, vertebrates) with nearly 30,000 species, covering about 96% of all extant fish species. They are ubiquitous throughout freshwater and marine environments from the deep sea to the highest mountain streams. In this class are included all the important commercial and recreational fishes (Encyclopaedia 2009).

Teleosts have a movable maxilla and premaxilla, and corresponding modifications in the jaw musculature occur. These modifications make it possible for teleosts to protrude their jaws outwards from the mouth. The caudal fin is homocercal, meaning the upper and lower lobes are about equal in size. The spine ends at the caudal peduncle, distinguishing this group from those in which the spine extends into the upper lobe of the caudal fin (Benton 1990).

6.1 By Size

The smallest fish species is *Paedocypris progenetica*, a type of minnow which lives in the dark-coloured peat swamps of the Indonesian island of Sumatra. The females of this species have a standard length of 7.9 mm (0.31 in) at maturity. Until recently, this was the smallest of all known vertebrates. However, recently a minute Papua New Guinea frog, *Paedophryne amauensis*, with a standard length of 7.7 mm (0.30 in) was discovered (Tamare 2012). The slender Indonesian fish may still be the smallest vertebrate by weight. Male individuals of the anglerfish species *Photocorynus spiniceps* are 6.2–7.3 mm long at maturity, and thus could be claimed as an even smaller species. However, these males do not survive on their own merits but only by sexual parasitism on the larger female (Ronald 2006). Another very small fish is the stout infant fish, a type of goby. According to the Guinness Book of World Records, the sinarapan, also a goby, is the world's smallest commercially harvested fish (Foot 2000). Found in the Philippines, they have an average length of 12.5 mm (0.49 in) and are threatened by overfishing (Froese and Pauly 2006).

The largest fish is the whale shark. It is a slow-moving filter feeding shark with a maximum length of 20 m (66 ft) and a maximum weight of 34 tonnes. Whale sharks can live up to 70 years (Froese and Pauly 2009).

The heaviest bony fish is the ocean sunfish. It can weigh upto 2,300 kg (5,100 lb). It is found in all warm and temperate oceans. The longest bony fish is the king of herrings. Its total length can reach 11 m (36 ft), and it can weigh up to 272 kg (600 lb). It is a rarely seen oarfish found in all the world's oceans, at depths between 20 m (66 ft) and 1,000 m (3,300 ft) (Froese and Pauly 2009).

6.2 By Life Span

Some of the shortest-lived species are gobies, which are small coral reef-dwelling fish. Some of the longest-lived are rockfish. The shortest-lived fish is the seven-figure pygmy goby, which lives for at most 59 days. This is the shortest lifespan for any vertebrate (Depczynski and Bellwood 2005). Short-lived fishes have particular value in genetic studies on aging. In particular, the ram cichlid is used in laboratory studies because of its ease of breeding and predictable aging pattern (Herrera and Jagadeeswaran 2004; Froese and Pauly 2009). The longest-lived fish is the rougheye rockfish *Sebastes aleutianus* (205 years), found offshore in the North Pacific at 25–900 m (14–490 fathoms). This fish exhibits negligible senescence. There are stories about Japanese koi goldfish passed from generation to generation for 300 years. Scientists are skeptical. Counting growth lines on the scales of fish confined to ponds or bowls is unreliable, since they lay down extra lines. The maximum reported age for a goldfish is 41 years.

The longest-living commercial fish may be orange, roughly with a maximum reported age of 149 years. One of the longest-living sport fish is the Atlantic tarpon, with a maximum reported age of 55 years. Some of the longest-living fish are living fossils, such as the green sturgeon. This species is among the longest-living species found in freshwater, with a maximum reported age of 60 years. They are also among the largest fish species found in freshwater, with a maximum reported length of 2.5 m. Another living fossil is the Australian lungfish. One individual has lived in an aquarium for 75 years and is the oldest fish in captivity. According to fossil records, the Australian lungfish has hardly changed for 380 million years. Among gobies, small coral reef-dwelling fishes are some of the shortest-lived fishes with the seven-figure pygmy goby living at most for 59 days.

6.2.1 By Habitat

There is 10,000 times more saltwater in the oceans than there is freshwater in the lakes and rivers. However, only 58% of extant fish species are saltwater. A disproportionate 41% are freshwater fish (the remaining 1% are anadromous) (Cohen 1970). This diversity in freshwater species is, perhaps, not surprising, since the thousands of separate lake habitats promote speciation.

Fish can also be demersal or pelagic. Demersal fishes live on or near the bottom of oceans and lakes, while pelagic fishes inhabit the water column away from the bottom. Habitats can also be vertically stratified. Epipelagic fishes occupy sunlit waters down to 200 m (110 fathoms), mesopelagic fish occupying deeper twilight waters down to 1,000 m (3,300 ft) and bathypelagic fish inhabiting the cold and pitch black depths below. Most oceanic species (78 or 44% of all fish species) live near the shoreline. These coastal fish live on or above the relatively shallow continental shelf. Only 13% of all fish species live in the open ocean, off the shelf. Of these, 1% are epipelagic, 5% are pelagic and 7% are deep water (Cohen 1970). Fishes are found in nearly all natural aquatic environments (Cohen 1970). Most fish, whether by species count or abundance, live in warmer environments with relatively stable temperatures. However, some species survive temperatures up to 44.6°C (112.3°F), while others cope with colder waters; there are over 200 finfish species south of the Antarctic Convergence. Some fish species tolerate salinities over 10% (Bone and Moore 2008). The world's deepest living fish, *Abyssobrotula galatheae*, a species of cusk eel, lives in the Puerto Rico Trench at a depth of 8,372 m (27,467 ft) (Bone and Moore 2008; Froese and Pauly 2009). At the other extreme, the Tibetan stone loach lives at altitudes over 5,200 m (17,100 ft) in the Himalayas. Some marine pelagic fish range over vast areas, such as the blue shark that lives in all oceans. At the other extreme are fish confined to single, small living spaces, such as isolated cave fish like *Lucifuga* in

the Bahamas and Cuba, or equally isolated desert pupfish living in small desert spring systems in Mexico and the southwest USA, or bythitid vent fish like *Thermichthys hollisi*, living around thermal vents 2,400 m (1,300 fathoms) down.

By Breeding Behaviour

Grouper are protogynous hermaphrodites, who school in harems of 3–15 females. When no male is available, the most aggressive and largest females shift sex to male, probably as a result of behavioural triggers. In very deep waters, it is not easy for a fish to find a mate. There is no light, so some species depend on bioluminescence. Others are hermaphrodites, which doubles their chances of producing both eggs and sperm when an encounter does occur (Ryan 2007). The female anglerfish releases pheromones to attract tiny males. When a male finds her, he bites on to her and never lets go. When a male of the anglerfish species *Haplophryne mollis* bites into the skin of a female, he releases an enzyme that digests the skin of his mouth and her body, fusing the pair to the point where the two circulatory systems join up. The male then atrophies into nothing more than a pair of gonads. This extreme sexual dimorphism ensures that, when the female is ready to spawn, she has a mate immediately available (Pietsch 2009). Some sharks, such as hammerheads are able to breed parthenogenetically.

By Brooding Behaviour

Fishes adopt a variety of strategies for nurturing their brood. Sharks, for example, variously follow three protocols with their brood. Most sharks, including lamniformes (Froese and Pauly 2006) are ovoviviparous, bearing their young after they nourish themselves after hatching and before birth, by consuming the remnants of the yolk and other available nutrients. Some such as hammerheads are viviparous, bearing their young after nourishing hatchlings internally, analogously to mammalian gestation. Finally, catsharks and others are, oviparous, laying their eggs to hatch in the water. Some animals, predominantly fish such as cardinal fish practice mouth brooding, caring for their offspring by holding them in the mouth of a parent for extended periods of time. Mouth brooding has evolved independently in several different families of fish.

Others, such as seahorse males, practice pouch-brooding, analogous to Australia's kangaroos, nourishing their offspring in a pouch in which the female lays them.

By Feeding Behaviour

There are three basic methods by which food is gathered into the mouths of fish: by suction feeding, by ram feeding and by manipulation or biting (Liem 1980). Nearly all fish species use one of these styles and most use two (Bone and Moore 2008).

7 Early Fish

Early fish lineages had inflexible jaws limited to little more than opening and closing. Modern teleosts have evolved protrusible jaws that can reach out to engulf prey (Liem 1980; Lauder 1980). An extreme example is the protrusible jaw of the slingjaw wrasse. Its mouth extends into a tube half as long as its body, and with a strong suction it catches prey. The equipment tucks away under its body when it is not in use (Froese and Pauly 2009; Bone and Moore 2008). In practice, feeding modes lie on a spectrum, with suction and ram feeding at the extremes. Many fish capture their prey using both suction pressures combined with a forward motion of the body or jaw (Norton and Bainerd 1993).

The cookiecutter shark is a small dogfish which derives its name from the way it removes small circular plugs, looking as though cut with a cookie cutter, from the flesh and skin of cetaceans and larger fish, including other sharks. The cookiecutter attaches to its larger prey with its suctorial lips and then protrudes its teeth to remove a symmetrical scoop of flesh. Most fishes are food opportunists or generalists. They eat whatever is most easily available. For example, the blue shark feeds on dead whales and nearly everything else that wriggles: other fish, cephalopods, gastropods, ascidians and

crustaceans. Ocean sunfishes prefer jellyfish (Froese and Pauly 2009).

Other fishes have developed extreme specialisations. Silver arowana, also called *monkey fish*, can leap 2 m out of the water to capture prey. They usually swim near the surface of the water waiting for potential prey. Their main diet consists of crustaceans, insects, smaller fishes and other animals that float on the water surface, for which their drawbridge-like mouth is exclusively adapted for feeding. The remains of small birds, bats and snakes have also been found in their stomachs (Froese and Pauly 2009). Archerfishes prey on land-based insects and other small animals by literally shooting them down with water droplets from their specialised mouths. Doctor fishes (*nibble fish*) live and breed in the outdoor pools of some Turkish spas, where they feed on the skin of patients with psoriasis. The fishes are like cleaner fish in that they only consume the affected and dead areas of the skin, leaving the healthy skin to recover.

7.1 By Vision

Four-eyed fishes have eyes raised above the top of the head which is divided into two different parts, so that they can see below and above the water surface at the same time. Four-eyed fishes actually have only two eyes, but their eyes are specially adapted for their surface-dwelling lifestyle. The eyes are positioned on the top of the head, and the fish floats at the water surface with only the lower half of each eye underwater. The two halves are divided by a band of tissue and the eye has two pupils, connected by part of the iris. The upper half of the eye is adapted for vision in air, the lower half for vision in water (Nelson 2006). The lens of the eye also changes in thickness top to bottom to account for the difference in the refractive indices of air versus water. These fishes spend most of their time at the surface of the water. Their diet mostly consists of the terrestrial insects which are available at the surface.

Many species of fish can see the ultraviolet end of the spectrum, beyond the violet (Jacobs 1992). The two-stripe damselfish, *Dascyllus reticulatus*, has ultraviolet-reflecting colouration which they appear to use as an alarm signal to other fish of their species. Predatory species cannot see this if their vision is not sensitive to ultraviolet. There is further evidence for this view that some fish use ultraviolet as a 'high-fidelity secret communication channel hidden from predators', while yet other species use ultraviolet to make social or sexual signals. Mesopelagic fishes live in deeper waters, in the twilight zone down to depths of 1,000 m, where the amount of sunlight available is not sufficient to support photosynthesis. These fishes are adapted for an active life under low light conditions. Barrel eyes are a family of small, unusual-looking mesopelagic fishes, named for their barrel-shaped, tubular eyes which are generally directed upwards to detect the silhouettes of available prey (Robison and Reisenbichler 2008).

The four-eyed fish feeds at the surface of the water with eyes that allow it to see both above and below the surface at the same time. The two-stripe damselfish can signal secret alarms by reflecting ultraviolet to other fish of its species. The barrel eye has barrel-shaped, telescopic eyes which are generally directed upwards, but can also be swirled forward. Flashlight fish uses a retroreflector behind the retina with photophores to detect eyeshine in other fish. Another mesopelagic fish is the flashlight fish. For more sensitive vision in low light, this fish has a retroreflector behind the retina. They also have photophores, which they use in combination with their retroreflector to detect eyeshine in other fish (Morin et al. 1975; McCosker 1977).

7.1.1 By Locomotion

The slowest-moving fishes are the sea horses. The slowest of these, the dwarf seahorse, attains about five feet per hour. Among the fastest sprinters are the Indo-Pacific sailfish and the black marlin. Both have been recorded in a burst at over 110 km per hour (68 mph). For the sailfish, that is equivalent to 12–15 times their own length per second. The wahoo is perhaps the

fastest fish for its size, attaining a speed of 19 lengths per second, reaching 78 km per hour.

The shortfin mako shark is fast enough and agile enough to chase down and kill an adult swordfish, but they do not always win. Sometimes in the struggle with a shark, a swordfish can kill it by ramming it in the gills or belly. This shark is highly migratory. Its exothermic constitution partly accounts for its relatively great speed (Passarelli et al. 2008). The Atlantic bluefin tuna is capable of sustained high speed cruising and maintains high muscle temperatures so it can cruise in relatively cold waters. The slowest fishes are the seahorses, and the tiny dwarf seahorse is the slowest of all. One of the fastest sprinters is the Indo-Pacific sailfish. The Atlantic bluefin tuna is capable of sustained high speed cruising.

7.1.2 Flying Fish

A number of species jump while swimming near the surface, skimming the water. Flying fishes have unusually large pectoral fins, which enable the fish to take short gliding flights above the surface of the water, in order to escape from predators. Their glides are typically around 50 m (160 ft), but they can use updrafts at the leading edge of waves to cover distances of at least 400 m (1,300 ft).

By Toxicity

Toxic fishes produce strong poisons in their bodies. Both poisonous fish and venomous fish contain toxins, but deliver them differently. Venomous fishes bite, sting or stab, causing an envenomation. Venomous fishes do not necessarily cause poisoning if they are eaten, since the digestive system often destroys the venom. By contrast, the digestive system does not destroy poisonous fish toxins, making them poisonous to eat. The most poisonous fish is the puffer fish. It is the second-most poisonous vertebrate after the golden dart frog. It paralyses the diaphragm muscles of human victims, who can die from suffocation. In Japan, skilled chefs use parts of a closely related species, the blowfish to create a delicacy called 'fugu', including just enough toxin for that 'special flavour'. The spotted trunkfish is a reef fish which secretes a colourless ciguatera toxin from glands on its skin when touched. The toxin is only dangerous when ingested, so there is no immediate harm to divers. However, predators as large as nurse sharks can die as a result of eating a trunkfish (Froese and Pauly 2009). The giant moray is a reef fish at the top of the food chain. Like many other apex reef fish, it is likely to cause ciguatera poisoning if eaten. Outbreaks of ciguatera poisoning in the eleventh to fifteenth centuries from large, carnivorous reef fish, caused by harmful algal blooms, could be a reason why Polynesians migrated to Easter Island, New Zealand and possibly Hawaii (Rongo et al. 2009). The puffer fish is the most poisonous fish in the world. The spotted trunkfish secretes a ciguatera toxin from glands on its skin. Like many other apex reef fish, the giant moray can cause ciguatera poisoning if eaten. A 2006 study found that there are at least 1,200 species of venomous fish (Smith and Wheeler 2006). There are more venomous fish than venomous snakes. In fact, there are more venomous fish than the combined total of all other venomous vertebrates. Venomous fishes are found in almost all habitats around the world, but mostly in tropical waters. They wound over 50,000 people every year. They carry their venom in venom glands and use various delivery systems, such as spines or sharp fins, barbs, spikes and fangs. Venomous fishes tend to be either very visible, using flamboyant colours to warn enemies, or skilfully camouflaged and maybe buried in the sand. Apart from the defence or hunting value, venom helps bottom-dwelling fish by killing the bacteria that try to invade their skin. The most venomous known fish is the reef stonefish (Froese and Pauly 2009). It has a remarkable ability to camouflage itself among rocks. It is an ambush predator that sits on the bottom waiting for prey to approach. Instead of swimming away if disturbed, it erects 13 venomous spines along its back. For defence, it can shoot venom from each or all of these spines. Each spine is like a hypodermic needle, delivering the venom from two sacs attached to the spine. The stonefish has control over whether to shoot its venom, and does so when provoked or

frightened (Grady 2006). The venom results in severe pain, paralysis and tissue death and can be fatal if not treated. Despite its formidable defences, stonefishes have predators. Some bottom-feeding rays and sharks with crushing teeth feed on them, as does the Stokes' seasnake.

Unlike stonefish, lionfish can only release venom when something strikes its spines. The stargazer buries itself and can deliver electric shocks as well as venom. It is a delicacy in some cultures (cooking destroys the venom) and can be found for sale in some fish markets with the electric organ removed. They have been called 'the meanest things in creation'(Grady 2006).

By Human Use

Fishes are sought by humans for their value as commercial food fish, recreational sport fish, decorative aquarium fish and, in tourism, attracting snorkelers and scuba divers. Throughout human history, important fisheries have been based on forage fish. Forage fishes are small fish which are eaten by larger predators. They usually school together for protection. Typical ocean forage fishes feed near the bottom of the food chain on plankton, often by filter feeding. They include the family Clupeidae (herrings, sardines, menhaden, hilsa, shad and sprats), as well as anchovies, capelin and halfbeaks. Important herring fisheries have existed for centuries in the North Atlantic and the North Sea. Likewise, important tradition for anchovy and sardine fisheries has operated in the Pacific, the Mediterranean and the southeast Atlantic (Bone and Moore 2008). The world annual catch of forage fish in recent years has been around 25 million tonnes, or one quarter of the world's total catch.

Higher in the food chain, Gadidae (cod, pollock, haddock, saithe, hake and whiting) also support important fisheries. Concentrated initially in the North Sea, Atlantic cod was one of Europe's oldest fisheries, later extending to the Grand Banks. Declining numbers led to international 'cod wars' and eventually the virtual abandonment of these fisheries. These days the Alaska pollock supports an important fishery in the Bering Sea and the north Pacific, yielding about six million tonnes, while cod amounts to about nine million tonnes (Bone and Moore 2008). Yellowfin tuna are now being fished as a replacement for the depleted Southern bluefin tuna.

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Biomass Production in Food Chain and Its Role at Trophic Levels

6

1 Introduction

The trophic level of an organism is the position it occupies in a food chain. The word trophic derives from the Greek τροφή (trophē) referring to food or feeding. A food chain represents a succession of organisms that eat another organism and are, in turn, eaten themselves. The number of steps an organism is from the start of the chain is a measure of its trophic level. Food chains start at trophic level 1 with primary producers such as plants, move to herbivores at level 2, predators at level 3 and typically finish with carnivores or apex predators at level 4 or 5. The path along the chain can form either a one-way flow or a food 'web'. Ecological communities with higher biodiversity form more complex trophic paths.

The organisms get food in three basic forms, that is, as producers, consumers and decomposers (Fig. 6.1):

- Producers (autotrophs) are plants or algae. These manufacture their own food by the process of photosynthesis and do not eat other organisms, and they are called as primary producers. The Sun's energy is used for the manufacture of food, and thus by the process of the food chain, transfer of energy takes place from lowest to the highest level (Butz 2002). However, in deep-sea hydrothermal ecosystems, no sunlight reaches the deep sea; therefore, manufacture of food by the primary producers is through a process which is called chemosynthesis (Dover 2000).
- Consumers (heterotrophs) are animals which cannot manufacture their own food and therefore they consume other organisms. Animals that eat primary producers (like plants) are called herbivores. Animals that eat other animals are called carnivores, and animals that eat both plant and other animals are called omnivores.
- Decomposers (detritivores) are those that break down dead plant and animal material and wastes and release it again as energy and nutrients into the ecosystem for the purpose of recycling. Decomposers are, namely, bacteria and fungi (mushrooms) that feed on waste and dead matter and convert it into inorganic chemicals that can be recycled as mineral nutrients for plants to be used again.

Trophic levels are generally represented by numbers, and they start from level 1 with plants. Further trophic levels are numbered one after the other according to how far the organism is along the food chain.

- Level 1: Plants and algae make their own food and are called primary producers.
- Level 2: Herbivores eat plants and are called primary consumers.
- Level 3: Carnivores that are eating herbivores are called secondary consumers.
- Level 4: Carnivores are generally those that eat other carnivores and are termed tertiary consumers.
- Level 5: Apex predators do not have predators and are at the top of the food chain.

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DOI 10.1007/978-81-322-0864-8_6,

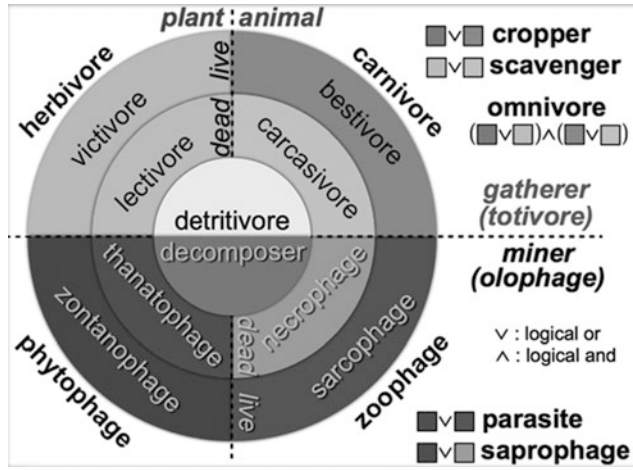

Fig. 6.1 Shows different levels of primary producers, primary, secondary and tertiary consumers and predators

There are number of food chains for almost all the organism present on the Earth, as most of the organisms are eaten by more than one kind of food or are eaten by more than one type of predator. A food web is that which overlaps or intersects food chains in an ecosystem. Decomposers are excluded from the food webs, but if they are included, they mark the end of a food chain (Lisowski et al. 2004). Thus, food chains start with primary producers and end up with the decomposers. Decomposers recycle all the nutrients, leaving them so they can be reused by primary producers, and they are sometimes regarded as occupying their own trophic level.

1.1 Biomass Transfer Efficiency

Each trophic level is related to the one below it by absorbing some of the energy it consumes, and in this way is supported by the next lower trophic level. Food chains generally show the amount of energy that moves from one feeding level to the next in a food chain. This is called an energy pyramid. The energy transferred between levels is thought to transfer in biomass, so energy pyramids can also be viewed as biomass pyramids as at higher levels biomass is consumed at lower levels (Fig. 6.2). The efficiency with which energy or biomass is transferred from one trophic level to the next is called the ecological efficiency. Consumers at each level convert on an average of only about 10% of the chemical energy in their food to their own organic tissue. For this reason, food chains rarely extend for more than 5 or 6 levels (American Heritage Science 2005).

2 Fractional Trophic Levels

Food webs are defined as ecosystems, and the trophic levels are the position of organisms within the webs. Trophic levels are not always simple integers because organisms often feed at more than one trophic level. For example, some carnivores also eat plants, and some plants are carnivores. A large carnivore may eat both smaller carnivores and herbivores; the bobcat eats rabbits, but the mountain lion eats both bobcats and rabbits. Animals can also eat each other; the bullfrog eats crayfish and crayfish eat young bullfrogs. The feeding habits of a juvenile animal, and consequently its trophic level, can change as it grows up. The fisheries scientist Daniel Pauly sets the values of trophic levels to one in plants and detritus, two in herbivores and detritivores (primary consumers), three in secondary consumers and so on. The definition of the trophic level, TL, for any consumer species is (Pauly and Palomares 2005)

$$\mathrm{TL}_i = 1 + \sum_j \left(\mathrm{TL}_i \cdot \mathrm{DC}_{ij}\right),$$

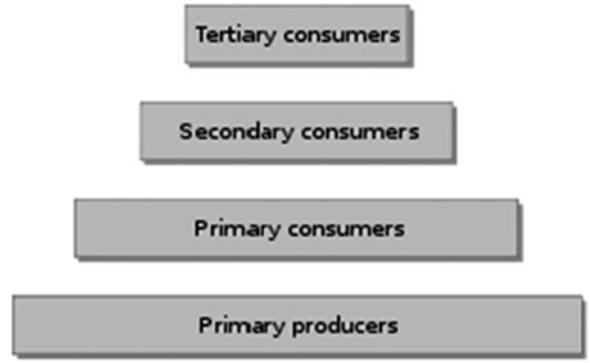

Fig. 6.2 An energy pyramid illustrates how much energy is needed to support the next trophic level

where TL_i is the fractional trophic level of the prey j and DC_{ij} represents the fraction of j in the diet of i. The values range in between 2.0 and 5.0 of most fish and other marine consumers in the case of marine ecosystems. The upper value, 5.0, is unusual, even for large fish (Cortés 1999), though it occurs in apex predators of marine mammals, such as polar bears and killer whales (Pauly et al. 1998a, b).

3 Mean Trophic Level

Due to overfishing of the tuna species, the mean trophic level of the world fisheries catch is declining at steady pace. In fisheries, the mean trophic level for the fisheries catch across an entire area or ecosystem is calculated for year y as

$$TL_y = \frac{\sum_i \left(TL_i \cdot Y_{iy}\right)}{\sum_i Y_{iy}},$$

where Y_{iy} is the catch of the species or group i in year y and TL_i is the fractional trophic level for species i as defined above (Pauly and Palomares 2005). Overfishing at the higher trophic levels results in higher economic values of fishes. There was a decline in level of fishes as the food web declined (Millennium Ecosystem Assessment 2005). But scientists have discovered that there is no relation between economic value and trophic level. The mean trophic levels in catches, surveys and stock assessments have in fact declined, suggesting that fishing down the food web is not a global phenomenon (Pauly et al. 2000).

3.1 FiB Index

Since biomass transfer efficiencies are only about 10%, it follows that the rate of biological production is much greater at lower trophic levels than it is at higher levels. Fisheries catches tend to increase as the trophic levels declines. The fisheries will target species lower in the food web (Pauly et al. 1998a, b). In 2000, this led Pauly and others to construct a 'Fisheries in Balance' index, usually called the FiB index. The FiB index is defined, for any year y, by Pauly and Palomares (2005):

$$FiB_y = \log\left(\left(Y_y \Big/ (TE)^{TL_y}\right) \Big/ \left(Y_0 \Big/ (TE)^{TL_0}\right)\right),$$

where Y_y is the catch at year y, TL_y is the mean trophic level of the catch at year y, Y_0 is the catch and TL_0 the mean trophic level of the catch at the start of the series being analysed and TE is the transfer efficiency of biomass or energy between trophic levels. The FiB index is stable (zero) over periods of time when changes in trophic levels are matched by appropriate changes in the catch in the opposite direction. The index increases if catches increase for any reason, for example, higher fish biomass or geographic expansion (Pauly and Palomares 2005). Such decreases explain the 'backward-bending' plots of trophic level versus catch originally observed by Pauly and others in 1998.

4 Trophic Dynamics

In ecology, trophic dynamics is the system of trophic levels which describes the position that an organism occupies in a food chain: what an organism eats and what eats the organism.

Ecologists study the energy economies of natural systems. Foundation species (also known as primary producers) harvest an energy source such as sunlight and turn it into biomass by fixing carbon dioxide. Organic compounds such as carbohydrates, fats and proteins are high-energy substances consumed by other organisms (primary consumers), which are in turn consumed by others. Each link in this chain of consumption is termed a trophic level. As only a fraction of the energy used by a level is converted to biomass, less energy is available at higher levels. Plants, algae and some bacteria can perform photosynthesis and combine water and carbon dioxide to make organic compounds using the Sun's energy.

Almost all the ecosystems rely upon the Sun for energy and upon autotrophs to fix carbon and harness that energy. Only a few bacteria, such as chemosynthetic archaea and bacteria, derive energy from the breakdown of sulphur compounds such as hydrogen sulphide around deep-sea hydrothermal vents and acid mine drainage. These organisms can utilise hydrogen sulphide in lieu of water to make organic compounds, and as the reaction between hydrogen sulphide and carbon dioxide is a spontaneous one, they do not need energy from sunlight. Lithotrophs can use inorganic compounds as electron donors to manufacture organic compounds or produce ATP. Sulphur-oxidising bacteria, for example, can consume hydrogen sulphide, elemental sulphur, sulphite and thiosulphate as energy sources instead of carbohydrates, fats and proteins. Using sulphite oxidase, sulphur-oxidising bacteria can obtain electrons from sulphur compounds and form ATP through an electron transport chain (ETC.).

In terrestrial ecosystems, plants such as grass are the primary producers and form the first trophic level. Next are herbivores (primary consumers) that eat the grass, such as rabbits. Next are carnivores (secondary consumers) that eat the rabbits, such as a bobcat. Every time there is an exchange of energy between one trophic level to another, there is quite a significant loss due to the fundamental laws of thermodynamics. Living organisms utilise energy from their food for cellular processes, growth and development. Animals need energy to move and digest food. A high metabolism also reduces the efficiency of the energy transfer by causing more energy to be lost as heat. Less energy is lost in the body of a fish than in the body of a small mammal. Energy is also excreted in urine and faeces. Therefore, so many units of grass can only support a much smaller number of units of rabbits, who can only support a smaller group of bobcats and mountain lions. This is why trophic levels are usually portrayed as a pyramid, one that places grass on the bottom and mountain lions on top. The top is generally much smaller than the bottom, although certain factors can produce an inverted pyramid if it is a pyramid of number or biomass. A pyramid of energy (which measures the energy or kilojoules) is never inverted. Each level implies a loss of energy and efficiency and less life that can be supported by the Sun.

There is no in-principle limit to the number of levels in a trophic system, but as only a fraction of the energy of each level can be processed by the next (about 10–20%), there are rarely more than four or five links of consumption.

5 Components of Ecosystems

Ecosystems have four basic components:

1. The abiotic environment
2. Producers
3. Consumers
4. Decomposers

Producers (autotrophs) utilise energy from the Sun and nutrients from the abiotic environment (carbon dioxide from the air or water, other nutrients from the soil or water, e.g. nitrates, phosphates and sulphates to manufacture proteins) to perform photosynthesis and grow. Producers are generally green plants (those with

chlorophyll). Autotrophs can be phototrophs (photoautotrophs) or lithotrophs (lithoautotrophs). Autotrophs are able to fix carbon dioxide and make their own organic compounds to be used for respiration and structural needs. Heterotrophs cannot fix carbon dioxide and require organic carbon for cell synthesis. Photoheterotrophs produce ATP through photophosphorylation, utilising light energy, and may not need an electron donor or acceptor. Photoheterotrophs must obtain carbon from organic compounds. Chemoheterotrophs can use either inorganic compounds (chemolithoheterotrophs) such as sulphur for energy or organic compounds (chemoorganoheterotrophs or simply organotrophs) such as carbohydrates, fats and proteins. Heterotrophs are generally consumers that feed on other organisms. Decomposers and detritivores utilise energy from wastes or dead organisms, and so complete the cycle by returning nutrients to the soil or water, and carbon dioxide to the air and water.

6 Biomass Production

Primary production is generation of biomass through photosynthesis. It can be measured in grams per square metre per year (g/m^2/year). The highest producers of biomass are:

- Swamps and marshes, 2,500 g/m^2/year of biomass
- Tropical rainforests, 2,000 g/m^2/year of biomass
- Algal beds and reefs, 2,000 g/m^2/year of biomass
- River estuaries, 1,800 g/m^2/year of biomass

Others include:

- Temperate forests, 1,250 g/m^2/year of biomass
- Cultivated lands, 650 g/m^2/year of biomass
- While lowest producers are deserts (3 g/m^2/year), open ocean (125 g/m^2/year) and tundra (140 g/m^2/year)

7 The Marine Food Chain

Predatory fish
↑
Filter feeders
↑
Predatory zooplankton
↑
Zooplankton
↑
Phytoplankton

In the ocean, phytoplankton is usually the primary producer. Phytoplankton converts inorganic carbon into protoplasm. Phytoplankton is consumed by microscopic animals called zooplankton (these are the second level in the food chain and include larval animals, such as young fish, squid and crab/lobster), as well as adult crustaceans called copepods.

Zooplankton is consumed both by other larger predatory zooplankters and by fish (the third level in the food chain). Fish that eat zooplankton could constitute the fourth trophic level, while seals consuming the fish are the fifth. Alternatively, for example, whales may consume zooplankton directly, leading to an environment with one less trophic level. Trophic levels are very similar on land, with plants being the first trophic level, cows eating the grass being the second and humans eating the cows being the third. The amount of biomass produced for a given amount of solar energy is highest at the first level. Less biomass is produced at the second level, for some energy is lost during the conversion. The more trophic levels there are, the more energy is lost. Humans are generally primary and secondary consumers and thus represent usually second and third trophic levels. Most humans are omnivores, which means they consume both plants and animals and therefore consume from different trophic levels. Consuming a vegetarian diet would mean eating at a lower trophic level and would cause less energy to be lost.

Each species in an ecosystem is affected by the other species in that ecosystem. There are very few single prey-single predator relationships. Most prey are consumed by more than one predator, and most predators have more than one prey. Their relationships are also influenced by other environmental factors. In most cases, if one species is removed from an ecosystem, other species will most likely be affected, in ways that may ultimately lead to extinction. Biodiversity can contribute to the stability of ecosystems, due to the diversity of functional responses of community members to perturbation. From the point of view of an individual organism, this can vary with different life history characteristics. For example, when an organism can exploit a wide range of resources, a decrease in biodiversity is often less likely to impact that organism. However, for an organism that can exploit only a limited range of resources, a decrease in biodiversity is more likely to have a strong effect. Reduction of habitat, hunting and fishing of some species to extinction or near extinction and eradication of insects and pollution tend to tip the balance of biodiversity. Similarly, in situ conservation areas are needed to be maintained for a diverse and stable environment for the threatened species to thrive.

8 Multitrophic Interactions

Multitrophic interactions are those that involve more than two trophic levels in a food web. The term is most often applied to interactions among plants, herbivores and predators. One example of a multitrophic interaction is a trophic cascade, in which predators help to increase plant growth and prevent overgrazing by suppressing herbivores. A simple way to show more than two trophic levels can be a pyramid, which shows the flow of energy throughout an ecosystem.

Ecologists collect data on trophic levels and food webs to statistically model and mathematically calculate parameters, such as those used in other kinds of network analysis, to study emergent patterns and properties shared among ecosystems. The emergent pyramidal arrangement of trophic levels with amounts of energy transfer decreasing as species become further removed from the source of production is one of several patterns that is repeated among the planets ecosystems. The size of each level in the pyramid generally represents biomass, which can be measured as the dry weight of an organism. Autotrophs may have the highest global proportion of biomass, but they are closely rivalled or surpassed by microbes.

Links in a food web illustrate direct trophic relations among species, but there are also indirect effects that can alter the abundance, distribution or biomass in the trophic levels. For example, predators eating herbivores indirectly influence the control and regulation of primary production in plants. Although the predators do not eat the plants directly, they regulate the population of herbivores that are directly linked to plant trophism. The net effect of direct and indirect relations is called trophic cascades. Trophic cascades are separated into species-level cascades, where only a subset of the food web dynamic is impacted by a change in population numbers, and community-level cascades, where a change in population numbers has a dramatic effect on the entire food web, such as the distribution of plant biomass.

9 Food Chain

A food chain shows how energy moves from one organism to another organism in an ecosystem. Autotrophs make their own food from inorganic carbon and provide a food source to primary consumers. The primary consumers grow and form their own organic compounds (e.g. proteins in muscle) from the plant food that they eat. These primary consumers store energy for consumers at higher levels. Trophic levels represent the flow of energy in food chains, with losses at each level. Food webs demonstrate more complex relationships between living organisms, as one animal may have several different food sources and several different predators.

The source of all food is the activity of autotrophs, mainly photosynthesis by plants.

Table 6.1 Food chain and trophic levels

Grass →	Grasshopper →	Toad →	Snake →	Hawk →	Bacteria of decay
In general,					
Autotrophs (producers) →	Herbivores (primary consumers) →	Carnivores (secondary, tertiary etc. consumers) →			Decomposers

- They are called producers because only they can manufacture food from inorganic raw materials.
- This food feeds herbivores, called primary consumers.
- Carnivores that feed on herbivores are called secondary consumers.
- Carnivores that feed on other carnivores are tertiary (or higher) consumers.

Such a path of food consumption is called a food chain.

Each level of consumption in a food chain is called a trophic level.

The table gives one example of a food chain and the trophic levels represented in it (Table 6.1).

9.1 Food Webs

Most food chains are interconnected. Animals typically consume a varied diet and, in turn, serve as food for a variety of other creatures that prey on them. These interconnections create food webs.

9.1.1 Energy Flow Through Food Chains

H. T. Odum analysed the flow of energy through a river ecosystem in Silver Springs, Florida.

At each trophic level, net production is only a fraction of gross production because the organisms must expand energy to stay alive. The difference between gross and net production is greater for animals than for the producers—reflecting their greater act.

Much of the energy stored in net production was lost to the system firstly by decay and secondly by being carried downstream. The substantial losses in net production as energy pass from one trophic level to the next. The ratio of net production at one level to net production at the next higher level is called the conversion efficiency. Here it varied from 17% from producers to primary consumers (1,478/8,833) to 4.5% from primary to secondary consumers (67/1,478). Similar studies carried out in other ecosystems, 10% of the average conversion efficiency is from producers to primary consumers. Animal husbandry often exceeds this 10% value. For example, broilers (young chickens) can gain half a pound (227 g) of weight for every pound (454 g) of food they eat. Nonetheless, the loss of energy as it passes from producers to primary consumers explains, for example, why it costs more to buy a pound of beefsteak than a pound of corn. Conversion efficiencies from primary consumers to secondary consumers (herbivores to carnivores) tend to be much lower, averaging about 1%. In this ecosystem, all the gross production of the producers (20,810) ultimately disappeared in respiration (14,198) and downstream export and decay (6,612). So there was no storage of energy from 1 year to the next. This is typical of mature ecosystems, such as a mature forest.

Some ecosystems do store energy, for example, the slow rate of decay in bogs causes peat to accumulate (the source of the world's coal). A young forest accumulates organic matter as the trees grow.

10 The Pyramid of Energy

Conversions efficiencies are always much less than 100%. At each link in a food chain, a substantial portion of the Sun's energy—originally trapped by a photosynthesising autotroph—is dissipated back to the environment (Fig. 6.3). Thus, it follows that the total amount of energy stored in the bodies of a given population is dependent on its trophic level. For example, the total amount of energy in a population of toads must necessarily be far less than that in the insects on which they feed. The insects, in turn, have only a fraction of the energy stored in the plants on which they feed. This decrease in the total available energy at each higher trophic level is called the pyramid of energy.

An energy pyramid is a graphical model of energy flow in a community. The different levels

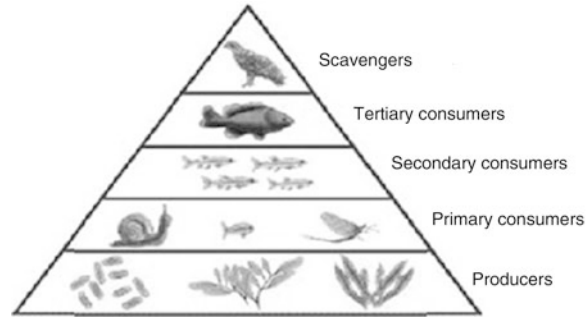

Fig. 6.3 Energy pyramid

represent different groups of organisms that might compose a food chain. From the bottom up, they are as follows:

- *Producers*—bring energy from nonliving sources into the community
- *Primary consumers*—eat the producers, which makes them herbivores in most communities
- *Secondary consumers*—eat the primary consumers, which makes them carnivores
- *Tertiary consumers*—eat the secondary consumers

In some food chains, there is a fourth consumer level and, rarely, a fifth. An energy pyramid's shape shows how the amount of useful energy that enters each level—chemical energy in the form of food—decreases as it is used by the organisms in that level. How does this happen? Cell respiration 'burns' food to release its energy and, in doing so, produces ATP, which carries some of the energy as well as heat, which carries the rest. ATP is then used to fuel countless life processes. The consequence is that even though a lot of energy may be taken in at any level, the energy that ends up being stored there—which is the food available to the next level—is far less. Scientists have calculated that an average of 90% of the energy entering each step of the food chain is 'lost' this way (although the total amount in the system remains unchanged). The consumers at the top of a food pyramid, as a group, thus have much less energy available to support them than those closer to the bottom. That is why their numbers are relatively few in most communities. Eventually, the amount of useful energy left cannot support another level. That is why energy flow is depicted in the shape of a pyramid. The energy that enters a community is ultimately lost to the living world as heat.

10.1 The Pyramid of Biomass

Since all organisms are made of roughly the same organic molecules in similar proportions, a measure of their dry weight is a rough measure of the energy they contain. A census of the population, multiplied by the weight of an average individual in it, gives an estimate of the weight of the population. This is called the biomass (or standing crop). This, too, diminishes with the distance along the food chain from the autotrophs which make the organic molecules in the first place.

Analysis of various ecosystems indicates that those with squat biomass pyramids are less likely to be disrupted by physical or biotic changes than those with tall, skinny pyramids.

Pyramid of biomass is the graphic representation of biomass present per unit area of different trophic levels with producers at the base and top carnivores at the tip (Fig. 6.4). The total amount of living or organic matter in an ecosystem at any time is called 'biomass'.

In a terrestrial ecosystem, the maximum biomass occurs in producers, and there is progressive decrease in biomass from lower to higher trophic levels. Thus, the pyramid of biomass in a terrestrial ecosystem is upright.

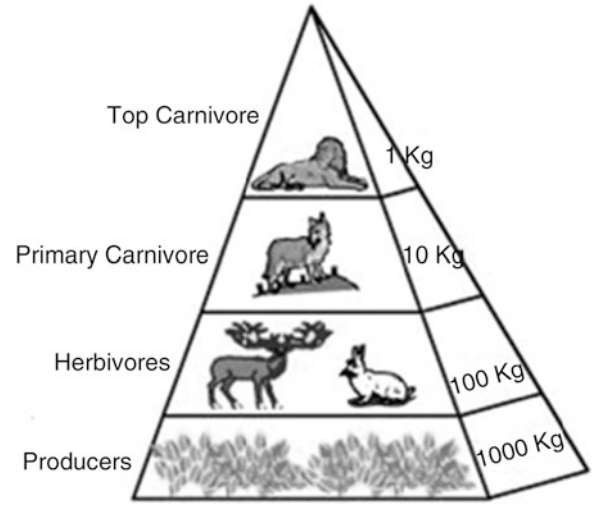

Fig. 6.4 Upright pyramid of biomass in a terrestrial ecosystem

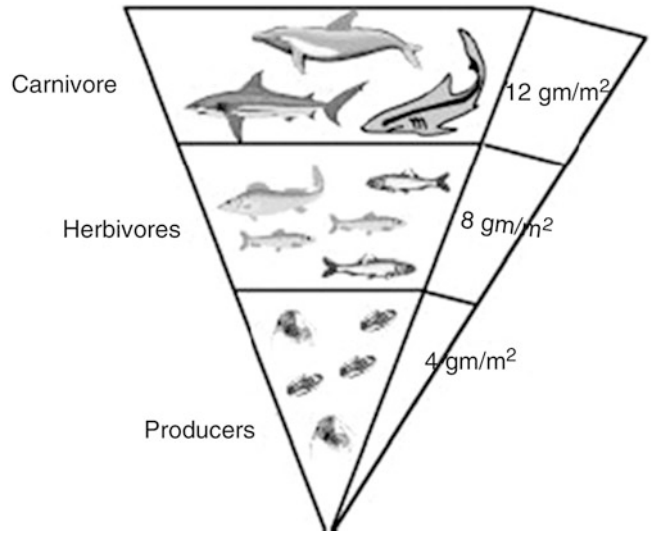

Fig. 6.5 Inverted pyramid in aquatic ecosystem

In an aquatic habitat, the pyramid of biomass is inverted or spindle shaped where the biomass of trophic level depends upon the reproductive potential and longevity of the member (Fig. 6.5).

10.1.1 The Pyramid of Numbers

Small animals are more numerous than larger ones. The pyramid of numbers results when a census of the populations of autotrophs, herbivores and two levels of carnivores was taken on an acre (0.4 ha) of grassland (Fig. 6.6). The pyramid arises because each species is limited in its total biomass by its trophic level. So, if the size of the individuals at a given trophic level is small, their numbers can be large and vice versa. Predators are usually larger than their prey. Occupying a higher trophic level, their biomass must be smaller. Hence, the number of individuals in the predator population is much smaller than that in the prey population. 'Pyramid of numbers is the graphic representation of number of individuals per unit area of various trophic levels stepwise with producers forming the base and top carnivores the tip'.

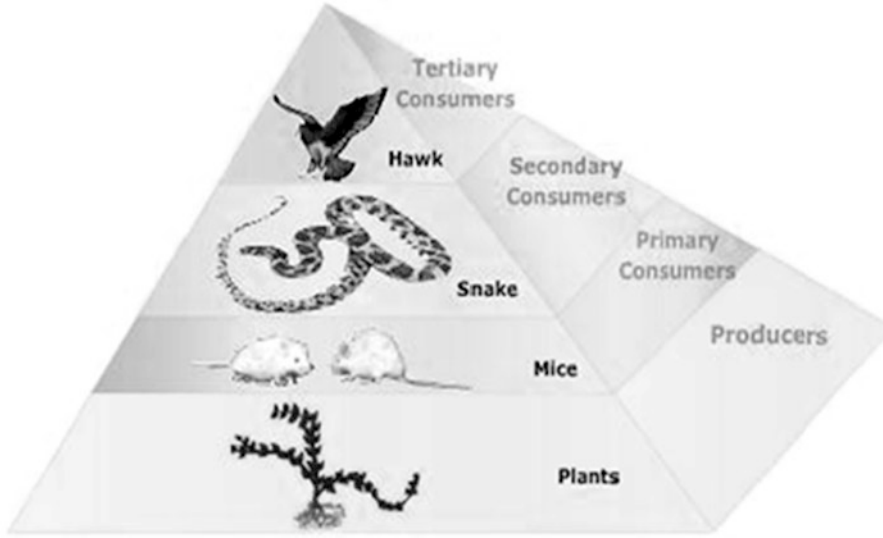

Fig. 6.6 Pyramid of numbers in a grassland ecosystem

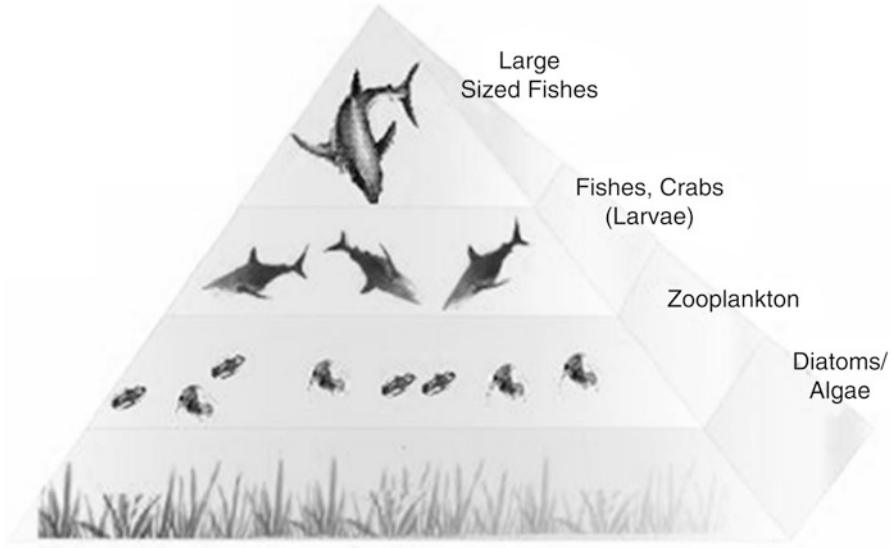

Fig. 6.7 Pyramid of numbers in an aquatic ecosystem

The shape of the pyramid of numbers varies from ecosystem to ecosystem.

In aquatic ecosystems and herbaceous communities, autotrophs are present in large numbers per unit area (Fig. 6.7). They support a lesser number of herbivores, which in turn support fewer carnivores.

So, the producers are smallest sized but maximum in number, while top carnivores are larger in size but lesser in number, so these cannot be used as prey by another. Hence, the pyramid of numbers is upright (Fig. 6.8).

In a parasitic food chain, for example, an oak tree, the large tree provides food to several

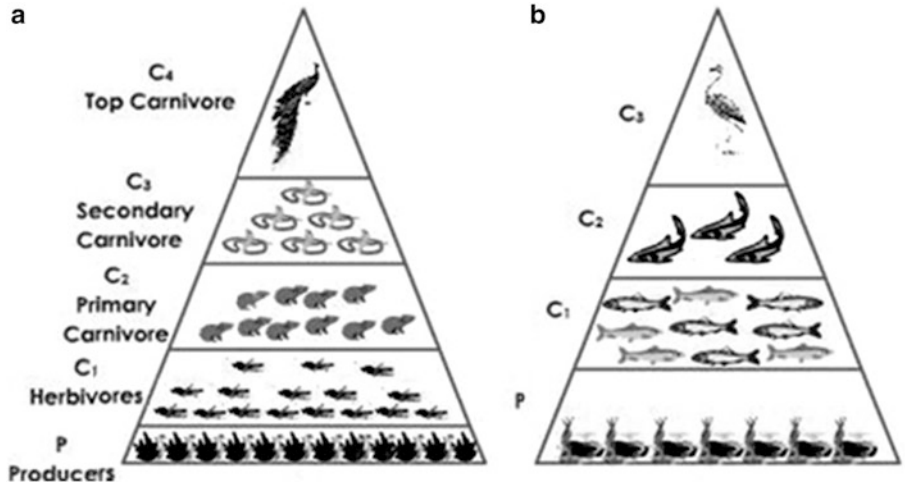

Fig. 6.8 Upright pyramids of numbers. (**a**) In a grass land and (**b**) in a pond

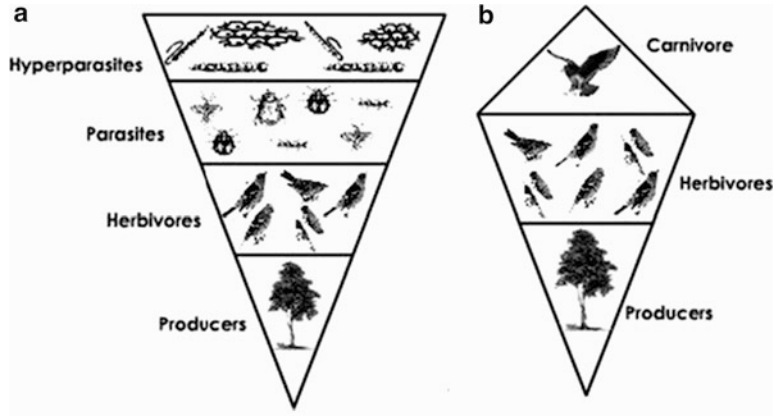

Fig. 6.9 Pyramid of numbers. (**a**) Inverted (**b**) Spindle shaped

herbivorous birds. The birds support still larger population of ectoparasites leading to the formation of an inverted pyramid (Fig. 6.9a).

When a large tree supports larger number of herbivorous birds which in turn are eaten by carnivorous birds like falcon and eagle, which are smaller in number, it forms a spindle-shaped pyramid (Fig. 6.9b).

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Concept of Rare and Endangered Species and Its Impact as Biodiversity

7

1 Introduction

In biology, a species is one of the basic units of biological classification and a taxonomic rank. A species is often defined as a group of organisms capable of interbreeding and producing fertile offspring. While in many cases this definition is adequate, the difficulty of defining species is known as the species problem. Differing measures are often used, such as similarity of DNA, morphology or ecological niche. Presence of specific locally adapted traits may further subdivide species into 'infraspecific taxa' such as subspecies. Species hypothesised to have the same ancestors are placed in one genus, based on similarities. The similarity of species is based on comparison of physical attributes, especially their DNA sequences, where available. All species are given a two-part name, a 'binomial name'. The first part of a binomial name is the generic name, the genus of the species. The second part is either called the specific name (a term used only in zoology) or the specific epithet (the term used in botany, which can also be used in zoology). For example, *Boa constrictor* is one of four species of the *Boa* genus. The first part of the name is capitalised, and the second part has a lower case. The binomial name is written in italics.

A usable definition of the word 'species' and reliable methods of identifying particular species are essential for stating and testing biological theories and for measuring biodiversity, though other taxonomic levels such as families may be considered in broad-scale studies (Sahney et al. 2010). Extinct species known only from fossils are generally difficult to assign precise taxonomic rankings, which is why higher taxonomic levels such as families are often used for fossil-based studies (Sahney et al. 2010; Sahney and Benton 2008). The total number of nonbacterial species in the world has been estimated at 8.7 million, with previous estimates ranging from 2 to 100 million.

It is surprisingly difficult to define the word 'species' in a way that applies to all naturally occurring organisms, and the debate among biologists about how to define 'species' and how to identify actual species is called the species problem. Over two dozen distinct definitions of 'species' are in use among biologists (Wilkins 2010).

Most textbooks follow Ernst Mayr's definition of a species as 'groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups' (de Queiroz 2005).

Various parts of this definition serve to exclude some unusual or artificial matings:

- Those that occur only in captivity (when the animal's normal mating partners may not be available) or as a result of deliberate human action
- Animals that may be physically and physiologically capable of mating but, for various reasons, do not normally do so in the wild

The typical textbook definition above works well for most multicelled organisms, but there

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are several types of situations in which it breaks down:

- By definition it applies only to organisms that reproduce sexually. So it does not work for asexually reproducing single-celled organisms and for the relatively few parthenogenetic multicelled organisms. The term 'phylotype' is often applied to such organisms.
- Biologists frequently do not know whether two morphologically similar groups of organisms are 'potentially' capable of interbreeding.
- There is considerable variation in the degree to which hybridisation may succeed under natural conditions or even in the degree to which some organisms use sexual reproduction between individuals to breed.
- In ring species, members of adjacent populations interbreed successfully but members of some nonadjacent populations do not.
- In a few cases it may be physically impossible for animals that are members of the same species to mate. However, these are cases, such as in breeds of dogs, in which human intervention has caused gross morphological changes and are therefore excluded by the biological species concept.

Horizontal gene transfer makes it even more difficult to define the word 'species'. There is strong evidence of horizontal gene transfer between very dissimilar groups of prokaryotes and at least occasionally between dissimilar groups of eukaryotes, and Williamson argues that there is evidence for it in some crustaceans and echinoderms. All definitions of the word 'species' assume that an organism gets all its genes from one or two parents that are very like that organism, but horizontal gene transfer makes that assumption false.

The question of how best to define 'species' is one that has occupied biologists for centuries, and the debate itself has become known as the species problem. Darwin wrote *On the Origin of Species*: No one definition has satisfied all naturalists, yet every naturalist knows vaguely what he means when he speaks of a species. Generally the term includes the unknown element of a distinct act of creation.

But later, in The Descent of Man, when addressing 'The question whether mankind consists of one or several species', Darwin revised his opinion to say, it is a hopeless endeavour to decide this point on sound grounds, until some definition of the term 'species' is generally accepted, and the definition must not include an element that cannot possibly be ascertained, such as an act of creation.

The modern theory of evolution depends on a fundamental redefinition of 'species'. Prior to Darwin, naturalists viewed species as ideal or general types, which could be exemplified by an ideal specimen bearing all the traits general to the species. Darwin's theories shifted attention from uniformity to variation and from the general to the particular. According to intellectual historian Louis Menand, 'Once our attention is redirected to the individual, we need another way of making generalizations. We are no longer interested in the conformity of an individual to an ideal type; we are now interested in the relation of an individual to the other individuals with which it interacts. To generalize about groups of interacting individuals, we need to drop the language of types and essences, which is prescriptive (telling us what finches should be), and adopt the language of statistics and probability, which is predictive (telling us what the average finch, under specified conditions, is likely to do). Relations will be more important than categories; functions, which are variable, will be more important than purposes; transitions will be more important than boundaries; sequences will be more important than hierarchies' (Menand 2001).

This shift results in a new approach to 'species'; Darwin concluded that species are what they appear to be: ideas, which are provisionally useful for naming groups of interacting individuals. 'I look at the term species', he wrote, 'as one arbitrarily given for the sake of convenience to a set of individuals closely resembling each other ... It does not essentially differ from the word variety, which is given to less distinct and more fluctuating forms. The term variety, again, in comparison with mere individual differences, is

also applied arbitrarily, and for convenience sake' (Menand 2001).

Practically, biologists define species as populations of organisms that have a high level of genetic similarity. This may reflect an adaptation to the same niche, and the transfer of genetic material from one individual to others, through a variety of possible means. The exact level of similarity used in such a definition is arbitrary, but this is the most common definition used for organisms that reproduce asexually (asexual reproduction), such as some plants and microorganisms.

This lack of any clear species concept in microbiology has led to some authors arguing that the term 'species' is not useful when studying bacterial evolution. Instead they see genes as moving freely between even distantly related bacteria, with the entire bacterial domain being a single gene pool. Nevertheless, a kind of rule of thumb has been established, saying that species of *bacteria* or *archaea* with 16S rRNA gene sequences more similar than 97% to each other need to be checked by DNA–DNA hybridisation if they belong to the same species or not (Stackebrandt and Goebel 1994). This concept has been updated recently, saying that the border of 97% was too low and can be raised to 98.7% (Stackebrandt and Ebers 2006).

In the study of sexually reproducing organisms, where genetic material is shared through the process of reproduction, the ability of two organisms to interbreed and produce fertile offspring of both sexes is generally accepted as a simple indicator that the organisms share enough genes to be considered members of the same species. Thus, a 'species' is a group of interbreeding organisms.

This definition can be extended to say that a species is a group of organisms that could potentially interbreed—fish could still be classed as the same species even if they live in different lakes, as long as they could still interbreed were ever they to come into contact with each other. On the other hand, there are many examples of series of three or more distinct populations, where individuals of the population in the middle can interbreed with the populations to either side but individuals of the populations on either side cannot interbreed. Thus, one could argue that these populations constitute a single species or two distinct species. This is not a paradox; it is evidence that species are defined by gene frequencies and thus have fuzzy boundaries.

Consequently, any single, universal definition of 'species' is necessarily arbitrary. Instead, biologists have proposed a range of definitions; which definition a biologists uses is a pragmatic choice, depending on the particularities of that biologist's research.

In practice, these definitions often coincide, and the differences between them are more a matter of emphasis than of outright contradiction. Nevertheless, no species concept yet proposed is entirely objective or can be applied in all cases without resorting to judgment. Given the complexity of life, some have argued that such an objective definition is in all likelihood impossible, and biologists should settle for the most practical definition.

For most vertebrates, this is the biological species concept (BSC) and to a lesser extent (or for different purposes) the phylogenetic species concept (PSC). Many BSC subspecies are considered species under the PSC; the difference between the BSC and the PSC can be summed up insofar as that the BSC defines a species as a consequence of manifest evolutionary history, while the PSC defines a species as a consequence of manifest evolutionary potential. Thus, a PSC species is 'made' as soon as an evolutionary lineage has started to separate, while a BSC species starts to exist only when the lineage separation is complete. Accordingly, there can be considerable conflict between alternative classifications based upon the PSC versus BSC, as they differ completely in their treatment of taxa that would be considered subspecies under the latter model (e.g. the numerous subspecies of honey bees).

1.1 Typological Species

A group of organisms in which individuals are members of the species if they sufficiently

conform to certain fixed properties or 'rights of passage'. The clusters of variations or phenotypes within specimens (i.e. longer or shorter tails) would differentiate the species. This method was used as a 'classical' method of determining species, such as with Linnaeus early in evolutionary theory. However, we now know that different phenotypes do not always constitute different species (e.g. a 4-winged *Drosophila* born to a 2-winged mother is not a different species). Species named in this manner are called morphospecies (Stackebrandt and Ebers 2006).

1.2 Evolutionary Species

A single evolutionary lineage of organisms within which genes can be shared and that maintains its integrity with respect to other lineages through both time and space. At some point in the evolution of such a group, some members may diverge from the main population and evolve into a subspecies, a process that may eventually lead to the formation of a new species if isolation (geographical or ecological) is maintained. A species that gives rise to another species is a paraphyletic species, or paraspecies.

1.3 Phylogenetic (Cladistic) Species

A group of organisms that shares an ancestor; a lineage that maintains its integrity with respect to other lineages through both time and space. At some point in the progress of such a group, members may diverge from one another: When such a divergence becomes sufficiently clear, the two populations are regarded as separate species. This differs from evolutionary species in that the parent species goes extinct taxonomically when a new species evolves, the mother and daughter populations now forming two new species (Ereshefsky 2002). Subspecies as such are not recognised under this approach; either a population is a phylogenetic species or it is not taxonomically distinguishable.

1.4 Other

1.4.1 Ecological Species

A set of organisms adapted to a particular set of resources, called a niche, in the environment. According to this concept, populations form the discrete phenetic clusters that we recognise as species because the ecological and evolutionary processes controlling how resources are divided up tend to produce those clusters.

1.4.2 Biological/Reproductive Species

Two organisms that are able to reproduce naturally to produce fertile offspring of both sexes. Organisms that can reproduce but almost always make infertile hybrids of at least one sex, such as a mule, hinny or F1 male cattalo, are not considered to be the same species.

1.4.3 Biological/Isolation Species

A set of actually or potentially interbreeding populations. This is generally a useful formulation for scientists working with living examples of the higher taxa like mammals, fish and birds, but more problematic for organisms that do not reproduce sexually. The results of breeding experiments done in artificial conditions may or may not reflect what would happen if the same organisms encountered each other in the wild, making it difficult to gauge whether or not the results of such experiments are meaningful in reference to natural populations.

1.4.4 Genetic Species

Based on similarity of DNA of individuals or populations. Techniques to compare similarity of DNA include DNA–DNA hybridisation and genetic fingerprinting (or DNA barcoding).

1.4.5 Cohesion Species

Most inclusive population of individuals having the potential for phenotypic cohesion through intrinsic cohesion mechanisms. This is an expansion of the mate-recognition species concept to allow for post-mating isolation mechanisms; no matter whether populations can hybridise

successfully, they are still distinct cohesion species if the amount of hybridisation is insufficient to completely mix their respective gene pools.

1.4.6 Evolutionarily Significant Unit (ESU)

An evolutionarily significant unit is a population of organisms that is considered distinct for purposes of conservation. Often referred to as a species or a *wildlife species*, an ESU also has several possible definitions, which coincide with definitions of species.

Morphological species a population or group of populations that differs morphologically from other populations. For example, one can distinguish between a chicken and a duck because they have different shaped bills and the duck has webbed feet. Species have been defined in this way since well before the beginning of recorded history. This species concept is highly criticised because more recent genetic data reveal that genetically distinct populations may look very similar, and, contrarily, large morphological differences sometimes exist between very closely related populations. Nonetheless, most species known have been described solely from morphology.

1.4.7 Phenetic Species

Based on phenotypes.

Microspecies: Species that reproduce without meiosis or fertilisation so that each generation is genetically identical to the previous generation.

Recognition Species: Based on shared reproductive systems, including mating behaviour. The recognition concept of species has been introduced by Hugh E. H. Paterson, after earlier work by Wilhelm Petersen.

Mate-Recognition Species: A group of organisms that are known to recognise one another as potential mates. Like the isolation species concept above, it applies only to organisms that reproduce sexually. Unlike the isolation species concept, it focuses specifically on pre-mating reproductive isolation.

1.5 Implications of Applying Species Status

The naming of a particular species may be regarded as a hypothesis about the evolutionary relationships and distinguishability of that group of organisms. As further information comes to hand, the hypothesis may be confirmed or refuted. Sometimes, especially in the past when communication was more difficult, taxonomists working in isolation have given two distinct names to individual organisms later identified as the same species. When two named species are discovered to be of the same species, the older species name is usually retained and the newer species name dropped, a process called synonymisation or, colloquially, as lumping. Dividing a taxon into multiple, often new, taxons is called splitting. Taxonomists are often referred to as 'lumpers' or 'splitters' by their colleagues, depending on their personal approach to recognising differences or commonalities between organisms.

Traditionally, researchers relied on observations of anatomical differences, and on observations of whether different populations were able to interbreed successfully, to distinguish species; both anatomy and breeding behaviour are still important to assigning species status. As a result of the revolutionary (and still ongoing) advance in microbiological research techniques, including DNA analysis, in the last few decades, a great deal of additional knowledge about the differences and similarities between species has become available. Many populations formerly regarded as separate species are now considered a single taxon, and many formerly grouped populations have been split. Any taxonomic level (species, genus, family, etc.) can be synonymised or split, and at higher taxonomic levels, these revisions have been still more profound.

From a taxonomical point of view, groups within a species can be defined as being of a taxon hierarchically lower than a species. In zoology only the subspecies is used, while in botany the variety, subvariety and form are used as well. In conservation biology, the concept of

evolutionary significant units (ESU) is used, which may define either species or smaller distinct population segments. Identifying and naming species is the providence of alpha taxonomy.

1.6 Endangered Species

An endangered species is a population of organisms which is at risk of becoming extinct because it is either few in numbers or threatened by changing environmental or predation parameters. Many nations have laws offering protection to conservation-reliant species, for example, forbidding hunting, restricting land development or creating preserves. Only a few of the many species is at risk of extinction actually make it to the lists and obtain legal protection like pandas. Many more species become extinct or potentially will become extinct, without gaining public notice.

2 Conservation Status

The conservation status of a species is an indicator of the likelihood of that endangered species not living. Many factors are taken into account when assessing the conservation status of a species, not simply the number remaining, but the overall increase or decrease in the population over time, breeding success rates, known threats and so on (Nature Serve 2012). Internationally, 199 countries have signed an accord agreeing to create biodiversity action plans to protect endangered and other threatened species.

3 Climate Change

Before greenhouse gases and global warming species were able to survive in their natural habitat, however, the rapid increase of climate change has put animals at risk of becoming extinct. Nigel Stork in the article 'Re-assessing Extinction Rate' explains, 'the key cause of extinction being climate change, and in particular rising temperatures, rather than deforestation alone'. Stork believes climate change is the major issue as to why species are becoming endangered. Stork claims rising temperature on a local and global level are making it harder for species to reproduce. As global warming continues, species are no longer able to survive and their progeny starts deteriorating. This is a repeating cycle that is starting to increase at a rapid rate because of climate change, therefore landing many species on the endangered species list.

4 IUCN Species at Risk of Extinction

The more general term used by the IUCN for species at risk of extinction is *threatened species*, which also includes the less-at-risk category of vulnerable species together with endangered and critically endangered. IUCN categories include:

1. *Extinct*: Examples are Javan Tiger, Thylacine, Dodo, Passenger Pigeon, Caribbean Monk Seal, Steller's Sea Cow, Aurochs, Elephant Bird, Woolly Mammoth and Dusky Seaside Sparrow.
2. *Extinct in the wild*: Captive individuals survive, but there is no free-living, natural population. Examples are Hawaiian Crow, Wyoming Toad, Socorro Dove, Red-tailed Black Shark, Scimitar Oryx and Catarina Pupfish.
3. *Critically endangered*: They face an extremely high risk of extinction in the immediate future. Examples are Mountain Gorilla, Bactrian Camel, Ethiopian Wolf, Saiga, Takhi, Kakapo, Arakan Forest Turtle, Sumatran Rhinoceros, Javan Rhino, Brazilian Merganser, Axolotl, Leatherback Sea Turtle, Northern White Rhinoceros, Gharial, Vaquita, Philippine Eagle, Brown Spider Monkey, California Condor, Island Fox, Black Rhinoceros and Chinese Alligator
4. *Endangered*: They face a very high risk of extinction in the near future. Examples are Dhole, Blue Whale, Asian Elephant, Giant Panda, Snow Leopard, African Wild Dog, Green Sea Turtle, Malayan Tapir, Tiger, Steller's Sea Lion, Philippine Eagle, Markhor,

Bornean Orangutan, Grevy's Zebra, Tasmanian Devil and Japanese Crane.

5. *Vulnerable*: They face a high risk of extinction in the medium term. Examples are African Elephant, Cheetah, Gaur, Lion, Sloth Bear, Dugong, Polar Bear, Indian Rhinoceros, Komodo Dragon, Great White Shark, Hippopotamus, Mandrill, Fossa and Crowned Crane.
6. *Near threatened*: They may be considered threatened in the near future. Examples are Blue-billed Duck, Solitary Eagle, American Bison, Jaguar, Maned Wolf, Tiger Shark, Southern White Rhinoceros, Okapi, African Grey Parrot, Striped Hyena and Narwhal.
7. *Least concern*: There is no immediate threat to the survival of the species. Examples are Common Wood Pigeon, Rock Pigeon, Giraffe, Common Bottlenose Dolphin, California Sea Lion, Brown Bear, Grey Wolf, House Mouse, Scarlet Macaw, Platypus, Human, Bald Eagle, Brown Rat, Cane Toad, Humpback Whale, Emperor Penguin, American Crow, Wolverine, Mute Swan, Mallard, Red-tailed Hawk, Indian Peafowl, American Alligator, Southern Elephant Seal, Meerkat

5 NatureServe Conservation Status

NatureServe and its member programmes and collaborators use a suite of factors to assess the conservation status of plant, animal and fungal species, as well as ecological communities and systems. These assessments lead to the designation of a conservation status rank. For species these ranks provide an estimate of extinction risk, while for ecological communities and systems, they provide an estimate of the risk of elimination. Conservation status ranks for ecological systems in North America are currently under development.

Conservation status ranks are based on a one to five scale, ranging from critically imperilled (G1) to demonstrably secure (G5). Status is assessed and documented at three distinct geographic scales—global (G), national (N) and state/province (S). The numbers have the following meaning:

- 1 = Critically imperilled
- 2 = Imperilled
- 3 = Vulnerable
- 4 = Apparently secure
- 5 = Secure

For example, G1 would indicate that a species is critically imperilled across its entire range (i.e. globally). In this sense the species as a whole is regarded as being at very high risk of extinction. A rank of S3 would indicate the species is vulnerable and at moderate risk within a particular state or province, even though it may be more secure elsewhere.

6 Impact on Biodiversity and Endangered Species

The first criterion to conserve the biodiversity of the planet is to take into consideration the reasons why so many species are becoming endangered. 'Habitat loss is the most widespread cause of species endangerment in the U.S., affecting 85% of imperiled species'. When an animal's ecosystem is not maintained, they lose their home and are either forced to adapt to new surroundings or perish. Pollution is another factor that causes many species to become endangered. Also, over-exploitation, disease and climate change have led to the endangerment of several species.

Humans have an impact on the species and their environment. 'As human use of resources, energy, and space intensified over the past few centuries, the diversity of life has been substantially diminished in most parts of the world'. Humans also have set standards for which species they think should be saved and which species they find unimportant or undesirable. For example, the coqui frog, an invasive species in Hawaii, is so common there that its 'nocturnal singing' reduces the value of homes and prevents hotels from using rooms near forests. Hawaiians have proposed eliminating the frog, and several wildlife managers want to release a pathogen to

kill the frogs. The frog has decreased the value of homes and caused a loss of business for several hotels, so the Hawaiians decided that it was acceptable to get rid of the group of coqui frog living near them. Another example where the human impact affected the welfare of a species sex is the instance of non-native mute swans establishing themselves at Arrowhead Lake in Vermont. When the population of swans grew to eight birds, the Vermont Fish and Wildlife Department decided to take action. Two swans were eventually killed, angering animal welfare organisations and people living near the lake.

7 Species Maintaining Importance

'Diversity of life and living systems are a necessary condition for human development'. Many question the importance of maintaining biodiversity in today's world, where conservation efforts prove costly and time-consuming. Species should be saved for 'aesthetic and moral justifications; the importance of wild species as providers of products and services are essential to human welfare. The value of particular species as indicators of environmental health or as keystone species is crucial to the functioning of ecosystems; and the scientific breakthroughs that have come from the study of wild organisms' Goldenberg (2011). In other words, species serve as a source of art and entertainment. They provide products such as medicine for human well-being, indicate the welfare of the overall environment and ecosystem, and provide research that resulted in scientific discoveries. An example of an 'aesthetic justification' in conserving endangered species is the introduction of the grey wolf into Yellowstone National Park. The grey wolf has brought numerous amounts of tourists to the park and added to the biodiversity in the protected region. Another example, supporting the conservation of endangered species as providers of products for human well-being, is the scrub mint. It has been found that the scrub mint contains an antifungal agent and a natural insecticide. The deterioration of the bald eagle and the peregrine falcon 'alerted people to the potential health hazards associated with the widespread spraying of DDT and other persistent pesticides'. This serves as an example of how certain fish can serve as identifiers of environmental health and protect human life as well as other species. Finally, an example of species providing for scientific discoveries is the instance of the Pacific yew which 'became the source of taxol, one of the most potent anticancer compounds ever discovered'. Endangered species could prove useful to human development, maintenance of biodiversity and preservation of ecosystems. Another approach is known as ecosystem conservation, where a focus is placed less on preserving any individual given species than on preserving the proper functioning of the ecosystem as a whole.

8 Preservation of Endangered Species

It is the goal of conservationists to create and expand upon ways to preserve endangered species and maintain biodiversity. There are several ways in which one can aid in preserving the world's species who are nearing extinction. One such way is obtaining more information on different groups of species, especially invertebrates, fungi and marine organisms, where sufficient data is lacking. For example, to understand the causes of population decline and extinction an experiment was conducted on the butterfly population in Finland. In this analysis, the butterflies' endangered list classification, distribution, density, larval specificity, dispersal ability, adult habitat breadth, flight period and body size were all recorded and examined to determine the threatened state of each species. It was found that the butterflies' distribution has declined by fifty-one and a half percent, and they have a severely restricted habitat. One example of specific butterflies who have a declining distribution rate are the Frigga's Fritillary and Grizzled Skipper, who have been affected by habitat loss due to extensive draining of the bogs where they live. This experiment shows that when one knows the

causes of endangerment, then one can successfully create solutions for the management of biodiversity.

Another way to help preserve endangered species is to create a new professional society dedicated to ecological ethics. This could help ecologists make ethical decisions in their research and management of biodiversity. Also, creating more awareness on environmental ethics can help to encourage species preservation. 'Courses in ethics for students, and training programs for ecologists and biodiversity managers' all could create environmental awareness and prevent violations of ethics in research and management. One final way in which one can conserve endangered species is through federal agency investments and protection enacted by the federal government. 'Ecologists have proposed biological corridors, biosphere reserves, ecosystem management, and ecoregional planning as approaches to integrate biodiversity conservation and socioeconomic development at increasingly larger spatial scales'. One example of a federal mandated conservation zone is the Northwest Hawaiian Islands Marine National Monument, the largest marine protected area in the world. The monument is essential for the preservation of underwater communities and overfished regions. Only researchers working in the area are permitted to fish, no corals may be removed and the Department of Homeland Security will enforce restrictions on vessels passing through the waters via satellite imaging. The monument will serve as a home to an estimated 7,000 species, most of which cannot be found anywhere else in the world. This environmental monument demonstrates the fact that it is possible to create a safe environment for endangered species, as well as maintaining some of the world's largest ecosystems.

9 Captive Breeding Programmes

Captive breeding is the process of breeding rare or endangered species in human controlled environments with restricted settings, such as wildlife preserves, zoos and other conservation facilities. Captive breeding is meant to save species from becoming extinct. It is supposed to stabilise the population of the species so it is no longer at risk for disappearing (Captive Breeding Populations 2009).

This technique has been used with success for many species for some time, with probably the oldest known such instances of captive mating being attributed to menageries of European and Asian rulers. However, captive breeding techniques are usually difficult to implement for highly mobile species like some migratory birds (e.g. cranes) and fishes (e.g. Hilsa). Additionally, if the captive breeding population is too small, inbreeding may occur due to a reduced gene pool; this may lead to the population lacking immunity to diseases.

10 Endangered Species in India

Endangered species in India include large varieties of rare species of flora and fauna. Indian wildlife that comprises numerous species of birds, animals, mammals, etc. is well famous for being one of the richest in the world. The Indian wildlife also contains several endangered species that are living critically on the verge of extinction. An endangered species is defined as a population of an organism that is at the danger of becoming extinct because of several reasons. Either they are few in number or are threatened by the varying environmental or predation parameters. The endangered species in India have been identified by different national and international organisations like the World Wildlife Fund (WWF), International Union for Conservation of Nature and Natural Resources (IUCN) and the Wildlife Institute of India (WII). As per the official records, in India, there are over 130,000 endangered animal species. However, some claim that the number is actually much more.

The increasing destruction of the natural habitat such as the biosphere reserves and tropical forests has posed a threat to the natural

endangered treasure. Mainly four reasons have been identified behind the extinction of endangered species in India. These are loss of a species as a biological entity, destabilisation of an ecosystem, endangerment of other species and loss of irreplaceable genetic material and associated biochemicals. When one species goes extinct, population increases or declines often result in secondary species. There is a possibility for an unstable spiral to arise, until other species are lost and the ecosystem structure is changed markedly and irreversibly. The endangered species in India have been divided into four main categories—critically endangered (CR), endangered (EN), vulnerable (VU) and threatened. This classification was done by the International Union for Conservation of Nature and Natural Resources (IUCN) and Wildlife Institute of India (WII), in the year 2004. The population of the endangered species has been decreasing every passing minute.

The number of endangered species in India accounts for around 8.86% of the world's mammals. The mammals are extended over 186 genera, 45 families and 13 orders out of which around 89 species are listed as threatened in the IUCN Red List of Threatened Animals (IUCN 2006). This also includes two species that are already locally extinct from India and the species are *Acinonyx jubatus* and *Rhinoceros sondaicus*. The mammals are actually the class of vertebrate animals, and they are mainly characterised by the presence of mammary glands, the presence of hair or fur, specialised teeth, the presence of a neocortex region in the brain and endothermic or 'warm-blooded' bodies. The mammals include nearly 5,500 species in the world. However, this varies with the classification scheme.

Among the endangered species in India, one of the most critically endangered one is the Siberian Tiger. This is a rare subspecies of tiger and they are an endangered species in India. The Asian Elephants found in India have also become the victims to the ever famous ivory poaching. However, their decline's main cause is considered to be the loss of habitat. According to the Elephants' Preservation Act, passed in India in the year 1879, 'no wild elephant shall be killed or captured unless in a person's self-defence, or because of damage being caused'. Another endangered species in India is one of the big cats, the Golden Leopard with black marks. The number of this species has been reduced to as low as 14,000, in India.

The main reasons behind the decline of leopard population in India have been the loss of habitat and also human population pressure on wildlife reserves in India. These reasons are also a matter of great concern for the other endangered species in India. The major reason behind the habitat loss is the spread of agriculture. The Bengal Tigers were also extensively being captured for pet trade, zoos and research, as well as for use in Oriental medicine, in the past. Further, the critically endangered species in India, as identified by the IUCN and WII, include the Jenkins Shrew, Malabar Large-spotted Civet, Namdapha Flying Squirrel, Pygmy Hog, Salim Ali's Fruit Bat, Sumatran Rhinoceros and the Wroughton's Free-tailed Bat. The list of endangered species in India include the Asiatic Lion, Asiatic Black Bear, Desert Cat, Great Indian Rhinoceros, Hispid Hare, Hoolock Gibbon, Kashmir Stag, Lion-tailed Macaque, Malabar Civet, Markhor, Nayan Ovis, Nilgiri Leaf Monkey, Pygmy Hog, Andaman Shrew, Andaman Spiny Shrew, Indian Elephant or Asian Elephant, Banteng, Blue Whale, Capped Leaf Monkey, Chiru, Fin Whale, Ganges River Dolphin, Golden Leaf Monkey, Hispid Hare, Asian Arowana, Loggerhead Sea Turtle, Hoolock Gibbon, Indus River Dolphin, Kondana Soft-furred Rat, Lion-tailed Macaque, Markhor, Marsh Mongoose, Nicobar Shrew, Nicobar Tree Shrew, Nilgiri Tahr, Parti-coloured Flying Squirrel, Peter's Tube-nosed Bat, Red Panda, Sei Whale, Servant Mouse, Snow Leopard, Tiger, Wild Water Buffalo and the Woolly Flying Squirrel.

Apart from the critically endangered and the endangered species in India, the International Union for Conservation of Nature and Natural Resources and Wildlife Institute of India also identified several species as vulnerable in India. These species include the Asiatic Wild Dog, Banteng Bos javanicus, Brow-antlered Deer, Brown Bear, Brown Palm Civet, Clouded Leopard, Common Otter, Ganges River Dolphin, Gaur, Goral,

Grey Indian Wolf, Himalayan White-toothed Shrew, Himalayan Musk Deer, Himalayan Shrew, Jackal Canis aureus, Andaman Horseshoe Bat, Andaman Rat, Argali, Asiatic Black Bear, Asiatic Golden Cat, Asiatic Wild Ass, Macaque Monkey, Back-striped Weasel, Barasingha, Bare-bellied Hedgehog, Blackbuck, Brown fish owl, Central Kashmir Vole, Dhole, Dugong, Eld's Deer, Elvira Rat, Eurasian Otter, Fishing Cat, Four-horned Antelope, Gaur, Himalayan Tahr, Humpback Whale, Indian Giant Squirrel, Irrawaddy Squirrel, Jerdon's Palm Civet, Kashmir Cave Bat, Kerala Rat, Khajuria's Leaf-nosed Bat, Kolar Leaf-nosed Bat, Lesser Horseshoe Bat, Mainland Serow, Malayan Porcupine, Mandelli's Mouse-eared Bat, Marbled Cat, Mouflon, Nicobar Flying Fox, Nilgiri Leaf Monkey, Nilgiri Marten, Nonsense Rat, Pale Grey Shrew, Palm Rat, Red Goral, Royal Bengal Tiger, Rock Eagle–Owl, Rusty-spotted Cat, Sikkim Rat, Sloth Bear, Slow Loris, Smooth-coated Otter, Sperm Whale, Sri Lankan Giant Squirrel, Sri Lankan Highland Shrew, Stump-tailed Macaque, Takin, Wild Goat, Wild Yak and the Lesser Panda. The species like the Indian Wild Ass, the Leopard and the Red Fox have been identified as the 'threatened species in India'.

11 Identifying of Species

Identifying species remains a controversial endeavour. Species debates persist on many fronts in evolutionary biology, ranging from philosophical exchanges about the biological 'reality' of species to fundamental disagreement about which operational concepts most closely reflect the processes by which new species arise. As these debates continue, conservation biologists and wildlife managers face a difficult and pressing challenge—they must decide what constitutes a 'good' species for conservation purposes and then apply these criteria to establish species boundaries in rare or threatened taxa.

Accurately defining species is critical to protecting biodiversity. Species continue to be the fundamental biological units that warrant legal protection under both national and international laws. When such laws are invoked, species boundaries determine the biological scope of all subsequent monitoring and recovery efforts. Hence, neglecting taxonomy can unwittingly lead to population declines and in some cases, complete species extinctions. Species boundaries can also serve to protect the larger ecosystems that endangered species occupy. Moreover, species are the common currency used to determine centres of endemism and biodiversity hotspots, geographic areas typically viewed as having the highest priority for protection. Hence, even conservation efforts focused on protecting ecosystems at regional or global scales rely heavily on how species are defined locally.

In recent reviews, scientists have argued that species propositions should be treated as biological hypotheses that can be explicitly tested. Yet practitioners attempting to evaluate species boundaries this way must contend with well over 25 unique species concepts, each emphasising different biological criteria that in some way characterise the overall process of evolutionary divergence. Species have been defined into four general categories: (1) phylogenetic species concepts emphasising shared evolutionary histories among populations; (2) similarity species concepts defined by common phenotypic features of the organisms, especially shared morphological traits; (3) ecological species concepts marked by adaptations to local environmental conditions; and (4) biological species concepts based on the ability of organisms to mate and produce viable offspring. Ideally, descriptions of species should address each of these four classes of concepts, but in practice this is seldom accomplished.

11.1 Methodology for Calculation of Rare and Endangered Species

Ideally, the definition of the environmental management class (EMC) should be based on

existing empirical relationships between flow changes and ecological status/conditions, which are associated with clearly identifiable thresholds. Therefore, EMC is a management concept that has been developed and used in the world because of a need to make decisions regardless of the limited lucid hydro-ecological knowledge available. The following are points that would go into determining the EMC for a particular river:

The rationale for ecological sensitivity and importance of river basin is that the higher the ecological sensitivity and importance of aquatic ecosystems in a river basin is, the higher the EMC should be, ideally. The more natural the current condition of the basin is, the greater the incentive for its maintenance as such. If the deterioration of aquatic environment continues, it will be more difficult to achieve a higher EMC, even if it is necessary, due to its high importance and sensitivity.

As this is the first time that such an approach is introduced in India, the focus would be on highlighting the main aquatic features and problems of each basin. This means that aggregate environmental indicators, which reflect different features or conditions of a river basin, could be used for scoring. Among the recent relevant works on this, the first question asked may be seen as an attempt to design a condensed measure of the ecological value of the basin, albeit in nonmonetary terms. An arbitrarily selected set of semi-quantitative and quantitative indicators includes:

- Presence of rare and endangered aquatic biota
- Presence of unique aquatic biota
- Diversity of aquatic habitats
- Presence of protected areas, areas of natural heritage and pristine areas, which are crossed by the main water course in the basin
- Sensitivity of aquatic ecosystems to flow reduction

Considering that most of the 'ecological' attention in countries like India has so far been given to fish, such indicators as rare and endangered biota and unique biota are calculated here using available fish data. Rare and endangered fish species are first identified using IUCN (1994) categories such as CR (critically endangered) and EN (endangered). Their cumulative number is then expressed as the proportion of the total number of fish species found in a river basin. The assessment of diversity of aquatic habitats and sensitivity of aquatic ecosystems to flow reduction requires expert judgment and knowledge of a particular river. Presence of protected or pristine areas can be assessed against existing guidelines for protected area management, that is, IUCN (1980), which sets the aim of 10% of the basin to be protected. The second question to be asked relates to what the river system looks like at present, compared to a reference condition in the past or compared to some similar and relatively undisturbed subbasins in the same physiographic settings. The indicators used in this study include:

- Percentage of the watershed remaining under natural vegetation cover types
- Percentage of the floodplain areas remaining under natural cover types
- Percentage of aquatic biota that are exotics
- Overall richness of aquatic species
- The degree of flow regulation
- The degree of river fragmentation
- Human population density in a river basin
- Percentage of population density in the main floodplains
- Overall water quality in the basin

The first two indicators are normally estimated from the GIS maps, remote sensing data or already published literature sources. In some cases, a percentage of the floodplain areas actually remaining in a basin compared to some past reference condition may be used as an alternative to the second indicator. A proportion of exotic species can be calculated as a percentage of the number of total fish species recorded in the basin. Overall species richness may be assessed as a proportion of the total number of species in a country; in a larger geographical region, whichever is more appropriate; or by an expert score on a scale from low to high. The most straightforward way of calculating the degree of flow regulation is as a ratio of total storage of all dams to the long-term mean annual natural flow volume of the basin. It is acknowledged though that this

approach does not recognise timing or types of flow events that are altered—which may be more critical than change in volume per se. A degree of river fragmentation can be represented by a simple indicator of spatial changes to habitat—longitudinal and latitudinal connectivity of rivers.

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Protected Areas in Relation to Marine Parks and Sanctuaries

8

1 Marine Parks

A marine park is a park consisting of an area of sea (or lake) sometimes protected for recreational use, but more often set aside to preserve a specific habitat and ensure the ecosystem is sustained for the organisms that exist there. Most marine parks are designed by government and organised like 'watery' national parks. The largest marine park is the Great Barrier Reef Marine Park in Australia, at 350,000 km^2. Although for many uses it is sufficient to designate the boundaries of the marine park and to inform commercial fishing boats and other maritime enterprises, some parks have gone to additional effort to make their wonders accessible to visitors. These can range from glass-bottomed boats and small submarines to windowed undersea tubes. In New Zealand a marine reserve is an area which has a higher degree of legal protection than marine parks for conservation purposes. In New South Wales, there are planned marine parks which will stretch along the coastline of the entire state.

2 Wildlife Sanctuaries

India is home to several fabulous wildlife sanctuaries and national parks, which makes this country a nature lover's paradise. The wildlife sanctuaries in India are home to around 2,000 different species of birds, 3,500 species of mammals, nearly 30,000 different kinds of insects and more than 15,000 varieties of plants. Travellers from all across the globe come to India to take a look at its rich wildlife and natural vegetation.

There are as many as 80 national parks and over 441 wildlife sanctuaries in India, covering nearly 4.5% of the total geographical area of the country. Scattered all across the country, these sanctuaries and parks attract the tourists with their beautiful landscapes, amazing rock formation and diverse range of flora and fauna. Most of these sanctuaries were originally private hunting grounds of the former Indian aristocratic families.

Paying a visit to India's wildlife sanctuaries is something that one should not rule out when on a holiday to this country. These sanctuaries and forest reserves are home to several endangered species of animals and birds like the Asiatic Elephant, the Royal Bengal tiger, the Snow Leopard and the Siberian Crane. Many of the forest reserves and wildlife sanctuaries of India are famous for some particular species of animals. For instance, the Kaziranga in Assam is known for the Indian Rhinoceros, while Periyar in Kerala is famous for its elephants. While embarking on a wildlife tour, travellers can pay a visit to the national parks in India. The Jim Corbett National Park, which is located in the Himalayan foothills, is the first of its kind. The Dudhwa National Park is another park famous by its huge swamp deer population.

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Tiger reserves are the best places to catch a glimpse of this big cat. The Kanha National Park in Madhya Pradesh is one of the largest tiger reserves of India.

The wildlife sanctuaries of India also include the bird sanctuaries, like the one at Bharatpur in Madhya Pradesh. The different species of birds that one can find over here is truly fascinating. Great Indian Bustard, Himalayan Monal Pheasant, Lammergeiers, Coughs, White-bellied Sea Eagle, White-breasted Swiftlet, Fruit Pigeons and Griffon Vultures are some of the bird species one gets to see here.

A trip to the wildlife sanctuaries in India brings one close to nature. The quiet and peaceful atmosphere of these parks can be enjoyed while walking down the trails or past the tall trees. A large number of wildlife safari tours are available which one can avail of in order to check out these wildlife sanctuaries.

3 Marine Protected Area

Marine protected areas, like any protected area, are regions in which human activity has been placed under some restrictions in the interest of conserving the natural environment, it's surrounding waters and the occupant ecosystems, and any cultural or historical resources that may require preservation or management. Marine protected areas' boundaries will include some area of ocean, even if it is only a small fraction of the total area of the territory.

Natural or historic marine resources are protected by local, state, territorial, native, regional or national authorities and may differ substantially from nation to nation. This variation includes different limitations on development, fishing practices, fishing seasons and catch limits, moorings and bans on removing or disrupting marine life of any kind.

In some situations, MPAs also provide revenue for countries, often of equal size as the income that they would have if they were to grant companies permissions to fish. As of 2010, the world hosted more than 6,800 MPAs, encompassing 1.17% of the world's oceans ('Global Ocean Protection: Present Status and Future Possibilities'. Iucn.org. 2010-11-23).

Marine protected areas are included on the World Database on Protected Areas (WDPA), since 2010, is viewable via Protected Planet, an online interactive search engine hosted by the United Nations Environment Programme's World Conservation Monitoring Centre (UNEP-WCMC).

3.1 Protected Areas

Protected areas are locations which receive protection because of their recognised natural, ecological and/or cultural values. There are several kinds of protected areas, which vary by level of protection depending on the enabling laws of each country or the regulations of the international organisations involved. The term 'protected area' also includes marine protected areas, the boundaries of which will include some area of ocean. There are over 161,000 protected areas in the world (as of October 2010) with more added daily, representing over 13% of the world's land surface area. By contrast, only 1.17% of the world's oceans is included in the world's ~6,800 marine protected areas.

Protected areas are essential for biodiversity conservation. They are the cornerstones of virtually all national and international conservation strategies. They are areas set aside to maintain functioning natural ecosystems, to act as refuges for species and to maintain ecological processes that cannot survive in most intensely managed landscapes and seascapes. Protected areas act as benchmarks against which we understand human interactions with the natural world. Today they are often the only hope we have of stopping many threatened or endemic species from becoming extinct.

Protected areas are designated with the objective of conserving biodiversity and providing an indicator for that conservation's progress, but the extent to which they defend resources and ecosystem dynamics from degradation are slightly more complex. Protected areas will usually encompass several other zones that have been

deemed important for particular conservation uses, such as Important Bird Areas (IBA) and Endemic Bird Areas (EBA), Centres of Plant Diversity (CBD), Indigenous and Community Conserved Areas (ICCA), Alliance for Zero Extinction Sites (AZE) and Key Biodiversity Areas (KBA). Likewise, a protected area or an entire network of protected areas may lie within a larger geographic zone that is recognised as a terrestrial or marine ecoregions, or a crisis ecoregions, for example.

Subsequently, the range of natural resources that any one protected area may guard is vast. They are allocated primarily for species conservation whether it be flora or fauna or the relationship between them. Protected areas are similarly important for conserving sites of cultural or indigenous importance and considerable reserves of natural resources.

3.2 Carbon Stocks

Carbon emissions from deforestation account for an estimated 20% of global carbon emissions, so in protecting the worlds carbon stocks greenhouse gas emissions are reduced and long-term land cover change is prevented, which is an effective strategy in the struggle against global warming. Of all global terrestrial carbon stock, 15.2% is contained within protected areas. Protected areas in South America hold 27% of the world's carbon stock, which is the highest percentage of any country in both absolute terms and as a proportion of the total stock.

3.3 Rainforests

18.8% of the world's forest is covered by protected areas, and 16 of the 20 forest types have 10% or more protected area coverage. Of the 670 ecoregions with forest cover, 54% have 10% or more of their forest cover protected under IUCN Categories I–VI.

3.4 Mountains

Nationally designated protected areas cover 14.3% of the world's mountain areas, and these mountainous protected areas make up 32.5% of the world's total terrestrial protected area coverage in 2009. Mountain protected area coverage has increased globally by 21% since 1990, and out of the 198 countries with mountain areas, 43.9% still have less than 10% of their mountain areas protected.

Annual updates on each of these analyses are made in order to make comparisons to the Millennium Development Goals and several other fields of analysis are expected to be introduced in the monitoring of protected areas management effectiveness, such as freshwater and marine or coastal studies which are currently under way, and islands and drylands which are currently in planning.

4 IUCN Protected Area Management Categories

Through its World Commission on Protected Areas (WCPA), IUCN have developed six Protected Area Management Categories that define protected areas according to their management objectives which are internationally recognised by various national governments and the United Nations. The categories provide international standards for defining protected areas and encourage conservation planning according to their management aims.

Recently, the importance of protected areas has been brought to the forefront at the threat of human-induced global warming and the understanding of the necessity to consume natural resources in a sustainable manner. The spectrum of benefits and values of protected areas is recognised not only ecologically, but culturally through further development in the arena of Indigenous and Community Conserved Areas (ICCAs). International programmes for the

protection of representative ecosystems remain relatively progressive, with less advances in marine and freshwater biomes.

Enforcing protected area boundaries is a costly and expensive, particularly if the allocation of a new protected region places new restrictions on the use of resources by the native people which may lead to their subsequent displacement. This has troubled relationships between conservationists and rural communities in many protected regions and is often why many wildlife reserves and national parks face the human threat of poaching for the illegal bush meat or trophy trades which is resorted to as an alternative form. How to manage areas protected for conservation brings up a range of challenges—whether it be regarding the local population, specific ecosystems or the design of the reserve itself—and because of the many unpredictable elements in ecology issues, each protected area requires a case specific set of guidelines.

There is an increasing and justifiable pressure to take proper account of human needs when setting up protected areas, and these sometimes have to be 'traded off' against conservation needs. Previously the government made decisions about protected areas and informed local people afterwards; today the emphasis is towards shifting the greater discussions with stakeholders and joint decisions about how such lands should be set aside and managed. Such negotiations are never easy but usually produce stronger and longer-lasting results for both conservation and people. In some countries, protected areas can be assigned without the infrastructure and networking needed to substitute consumable resources and protect the area from development or misuse. The soliciting of protected areas may require regulation to the level of meeting demands for food, feed, livestock and fuel, and the legal enforcement of not only the protected area itself but also 'buffer zones' surrounding it, which may help to resist destabilisation.

5 Coastal Zone

Central government has declared the coastal stretches of seas, bays, estuaries, creeks, rivers and back waters which are influenced by tidal action (in the landward side) up to 500 m from the High Tide Line (HTL) and the land between the Low Tide Line (LTL) and the HTL as 'Coastal Regulation Zone' (CRZ).

5.1 Classification Criteria and Regulatory Norms

The coastal regulation zone has been classified as CRZ-I, CRZ-II and CRZ-III in the state for the purpose of regulation of the permitted activities.

5.1.1 CRZ-I

Ecological sensitive area and the area between High Tide Line (HTL) and Low Tide Line (LTL). No new construction is permitted except for a few specified most essential activities like support activities for atomic energy plants and defence requirements, facilities required for disposal of treated effluents and other port-related waterfront activities.

5.1.2 CRZ-II

The area that has been developed up to or close to the shoreline which includes the designated urban areas that are substantially built up. Buildings permitted only on the landward side of the existing road (or roads approved in the Coastal Zone Management Plan of the area) or on the landward side of the existing authorised structures as defined in the notification. Reconstruction of the authorised buildings permitted subject to existing FSI/FAR norms without change in the use.

5.1.3 CRZ-III

The areas that are relatively undisturbed and those which do not belong to either CRZ-I or CRZ-II which includes mainly the rural area

and those not substantially built up within designated urban areas. The area up to 200 m from HTL is earmarked as 'No Development Zone'. No construction is permitted within this zone except for repairs to the existing authorised structures without exceeding existing FSI, plinth area and density. Development of vacant plots between 200 and 500 m of HTL is permitted in CRZ III for the purpose of construction of dwelling units and hotels/beach resorts subject to certain conditions.

6 Karnataka State Coastal Zone Management Plan

Coastal Zone Management Plan (CZMP) of the state was prepared and was approved by Ministry of Environment and Forest in the year 1996. According to this areas covered under CRZ-I, CRZ-II and CRZ-III were identified using satellite imagery and prepared maps in the scale 1:25,000. The enforcement of the law and the management of the zone from the point of view of protection of environment using these maps were difficult, the following steps are taken:

- Demarcation of High Tide Line (HTL) and Low Tide Line (LTL) on the ground and fixing reference pillars all along the coast and rivers
- Preparation of local level Coastal Regulation Zone Maps on the cadastral maps indicating HTL, LTL, 200 mt line, 100 mt line and other lines required for the purpose of enforcement of the law, the reference pillars and different zones of regulation

The above task has been entrusted to National Hydrographic Office, Dehradun, which is an organisation under the Ministry of Defence of Government of India and an agency authorised by Ministry of Environment of Forest for this purpose at an estimated cost of Rs.2.34 crores. This project is funded by KUIDFC under KUDCEMP. Demarcation and fixing of reference pillars is completed in Dakshina Kannada District, and the work is under progress in the other two districts. Preparation of local level maps for Dakshina Kannada District is in the final stage and mapping of other two districts has begun.

The coastline or seashore is where the land meets the sea or ocean. A precise line that can be called a coastline cannot be determined due to the dynamic nature of tides. The term 'coastal zone' can be used instead, which is a spatial zone where interaction of the sea and land processes occurs. Both the terms coast and coastal are often used to describe a geographic location or region, for example, New Zealand's West Coast, or the East and West Coasts of the United States. A pelagic coast refers to a coast which fronts the open ocean, as opposed to a more sheltered coast in a gulf or bay. A shore, on the other hand, can refer to parts of the land which adjoin any large body of water, including oceans (seashore) and lakes (lakeshore). Similarly, the somewhat related term 'bank' refers to the land alongside or sloping down to a river (riverbank) or to a body of water smaller than a lake. 'Bank' is also used in some parts of the world to refer to an artificial ridge of earth intended to retain the water of a river or pond. In other places this may be called a levee. While many scientific experts might agree on a common definition of the term 'coast', the delineation of the extents of a coast differ according to jurisdiction, with many scientific and government authorities in various countries differing for economic and social policy reasons.

Basically coasts are just beautiful tides which determine the range over which sediment is deposited or eroded. Areas with high tidal ranges allow waves to reach farther up the shore, and areas with lower tidal ranges produce deposition at a smaller elevation interval. The tidal range is influenced by the size and shape of the coastline. Tides do not typically cause erosion by themselves; however, tidal bores can erode as the waves surge up river estuaries from the ocean. Sediment deposited by rivers is the dominant influence on the amount of sediment located on a coastline. Today riverine deposition at the coast is often blocked by dams and other human regulatory devices, which remove the sediment from the stream by causing it to be deposited inland. Like the ocean which shapes them, coasts are a

dynamic environment with constant change. The Earth's natural processes, particularly sea level rises, waves and various weather phenomena, have resulted in the erosion, accretion and reshaping of coasts as well as flooding and creation of continental shelves and drowned river valleys (rias). Basically coasts are just beautiful. Tides often determine the range over which sediment is deposited or eroded. Areas with high tidal ranges allow waves to reach farther up the shore, and areas with lower tidal ranges produce deposition at a smaller elevation interval. The tidal range is influenced by the size and shape of the coastline. Tides do not typically cause erosion by themselves; however, tidal bores can erode as the waves surge up river estuaries from the ocean.

Waves erode coastline as they break on shore releasing their energy; the larger the wave, the more energy it releases and the more sediment it moves. Coastlines with longer shores have more room for the waves to disperse their energy, while coasts with cliffs and short shore faces give little room for the wave energy to be dispersed. In these areas the wave energy breaking against the cliffs is higher, and air and water are compressed into cracks in the rock, forcing the rock apart, breaking it down. Sediment deposited by waves comes from eroded cliff faces and is moved along the coastline by the waves. Sediment deposited by rivers is the dominant influence on the amount of sediment located on a coastline. Today riverine deposition at the coast is often blocked by dams and other human regulatory devices, which remove the sediment from the stream by causing it to be deposited inland. Like the ocean which shapes them, coasts are a dynamic environment with constant change. The Earth's natural processes, particularly sea level rises, waves and various weather phenomena, have resulted in the erosion, accretion and reshaping of coasts as well as flooding and creation of continental shelves and drowned river valleys. The coast and its adjacent areas on and off shore is an important part of a local ecosystem as the mixture of freshwater and saltwater in estuaries provides many nutrients for marine life. Salt marshes and beaches also support a diversity of plants, animals and insects crucial to the food chain. The high level of biodiversity creates a high level of biological activity, which has attracted human activity for thousands of years.

7 Human Impacts

7.1 Human Uses of Coasts

Once a fishing port, the harbour is now dedicated to tourism and pleasure boating. It can be seen that the sand and rocks have been darkened by oil slick up to the high-water line. An increasing part the global population inhabits coastal regions (Goudarzi 2006). Many of the world's major cities have been built on or near good harbours and have port facilities. Jurisdictions that are landlocked have achieved port status by such measures such as building canals. The coast is a crucial frontier that nations typically defended against military invaders, smugglers and illegal migrants. Fixed coastal defences have long been erected in many nations, and coastal countries typically have a navy and some form of coast guard. Coasts, especially those with beaches and warm water are an important draw for tourists. In many island nations such as those of the Mediterranean, South Pacific and Caribbean, tourism is central to the economy. Coasts are popular destinations because of recreational activities such as swimming, fishing, surfing, boating and sunbathing. Growth management can be a challenge for coastal local authorities who often struggle to provide the infrastructure required by new residents.

7.2 Threats to a Coast

Coasts also face many environmental challenges relating to human-induced impacts. The human influence on climate change is thought to be a contributing factor of an accelerated trend in sea level rise which threatens coastal habitat. Pollution can occur from a number of sources: garbage and industrial debris, the transportation of

petroleum in tankers, increasing the probability of large oil spills and small oil spills created by large and small vessels, which flush bilge water into the ocean. Fishing has diminished due to habitat degradation, overfishing, trawling, bycatch and climate change. Since the growth of global fishing enterprises after the 1950s, intensive fishing has gone from a few concentrated areas to encompass nearly all fisheries. The scraping of the ocean floor in bottom dragging is devastating to coral, sponges and other long-lived species that do not recover quickly. This destruction alters the functioning of the ecosystem and can permanently alter species composition and biodiversity. Bycatch, the capture of unintended species in the course of fishing, is typically returned the ocean only to die from injuries or exposure. Bycatch represents approximately one-fourth of all marine catch. In the case of shrimp capture, the bycatch is five times larger than the shrimp caught.

Also, the melting arctic ice will cause sea rise which will flood coastal areas.

8 Conservation

Extraordinary population growth in the twentieth century has placed stress on the planet's ecosystems. For example, on Saint Lucia, harvesting mangrove for timber and clearing for fishing drove the mangrove forests to low levels, resulting in a loss of habitat and spawning ground for marine life that was unique to the area. These forests also helped to stabilise the coastline. Conservation efforts since the 1980s have partially restored the ecosystem.

8.1 Types of Coast

According to one principle of classification, an emergent coastline is a coastline which has experienced a fall in sea level, because of either a global sea level change or local uplift. Emergent coastlines are identifiable by the coastal landforms, which are above the high tide mark, such as raised beaches. Alternatively, a submergent coastline is a coastline which has experienced a rise in sea level, due to a global sea level change, local subsidence or isostatic rebound. Submergent coastlines are identifiable by their submerged, or 'drowned' landforms, and fjords.

According to a second principle of classification, a concordant coastline is a coastline where bands of different rock types run parallel to the shore. These rock types are usually of alternating resistance, so the coastline forms distinctive landforms, such as coves. A discordant coastline is a type of coastline formed when rock types of alternating resistance run perpendicular to the shore. Discordant coastlines feature distinctive landforms because the rocks are eroded by ocean waves. The less resistant rocks erode faster, creating inlets or bays; the more resistant rocks erode more slowly, remaining as headlands or outcroppings.

8.2 Coastal Landforms

Animals living along the coast vary enormously; some live along coasts to nest like puffins, sea turtles and rockhopper penguins. Sea snails and various kinds of barnacles live on the coast and scavenge on food deposited by the sea. Most coastal animals are used to humans in developed areas, such as dolphins and seagulls who eat food thrown for them by tourists. Since the coastal areas are all part of the littoral zone, there is a profusion of marine life found just off-coast. There are many kinds of seabirds on the coast. Pelicans and cormorants join up with terns and oystercatchers to forage for fish and shellfish on the coast. There are also sea lions on the coast of Wales and other countries. Coastal areas are famous for their kelp beds. Kelp is a fast-growing seaweed that grows up to a metre a day. Corals and anemones are true animals but live a similar lifestyle as plants do. Mangroves and salt marsh are important coastal vegetation types in topical and temperate environments respectively.

8.3 The Coastline Problem

At some time in the years immediately preceding 1951, Lewis Fry Richardson in researching the possible effect of border lengths on the probability of war noticed that the Portuguese reported their measured border with Spain to be 987 km, but the Spanish reported it to be 1,214 km. This was the beginning of the coastline problem, which is how to arrive at an estimate of a boundary that is infinite.

Richardson's belief was based on Euclidean geometry that a coastline would approach a fixed length, as do similar estimations of regular geometric figures. For example, the perimeter of a regular polygon inscribed in a circle approaches the circumference with increasing numbers of sides (and decrease in the length of one side). In geometric measure theory such a smooth curve as the circle that can be approximated by small straight segments with a definite limit is termed a rectifiable curve.

8.4 Describing a Coastline

More than a decade after Richardson's work was finished, Benoît Mandelbrot invented a new branch of mathematics, fractal geometry, to describe just such non-rectifiable complexes in nature as the infinite coastline. A key property of the fractal is self-similarity; that is, at any scale the same general configuration appears. A coastline is perceived as bays alternating with promontories. No matter how greatly any one small section of coastline is magnified, a similar pattern of bays and promontories on bays and promontories appears, right down to the grains of sand. At that scale the coastline appears as a momentarily shifting, potentially infinitely long thread with a stochastic arrangement of bays and promontories formed from the small objects at hand. In such a real environment Mandelbrot asserts, 'coastline length turns out to be an elusive notion that slips between the fingers of those who want to grasp it'.

A coastline is definitely represented by a fractal. However, there are different kinds of fractals. A coastline is in 'a first category of fractals, namely curves whose fractal dimension is greater than 1'. That last statement represents an extension by Mandelbrot of Richardson's thought. Mandelbrot's statement of the Richardson Effect is

$$L(\varepsilon) \sim F\varepsilon^{1-D}$$

where L, coastline length, a function of the measurement unit, ϵ, is approximated by the expression. F is a constant and D is a parameter that Richardson found depended on the coastline approximated by L. He gave no theoretical explanation but Mandelbrot identified L with a non-integer form of the Hausdorff dimension, later the fractal dimension. Rearranging the right side of the expression obtains

$$\frac{F}{\varepsilon^D} \cdot \varepsilon$$

where $F\epsilon^{-D}$ must be the number of units ϵ required to obtain L. The fractal dimension is the number of the dimensions of the figure being used to approximate the fractal: 0 for a dot, 1 for a line and 2 for a square. D in the expression is between 1 and 2, for coastlines typically less than 1.5. The broken line measuring the coast does not extend in one direction nor does it represent an area, but is intermediate. It can be interpreted as a thick line or band of width 2ϵ. More broken coastlines have greater D and therefore L is longer for the same ϵ. Mandelbrot showed that D is independent of ϵ.

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Principles of Statistics and Reporting of Data

9

1 Introduction

Statics is the study of the collection, organisation and interpretation of data (Dodge 2003). It deals with all aspects, including the planning of data collection in terms of the design of surveys and experiments. Statistical analyst is one who is well versed in the ways of thinking and applies successfully the data for statistical analysis. The experience is gained by working in a number of fields. There is also a discipline called mathematical statistics, which is concerned with the theoretical basis of the subject.

2 Scope

Statistics is considered to be a mathematical science, and it pertains to the collection of data, analysis, interpretation or explanation and presentation of data (Moses 1986), while others consider it a branch of mathematics (Hay 1973). It is concerned mainly with collecting and interpreting data. As it has its empirical roots and its focus on applications, statistics is usually considered to be a distinct mathematical science rather than a branch of mathematics (Moore 1992; Chance et al 2005). Statisticians improve the quality of data with the designing of experiments and survey sampling. Statistics also provides tools for prediction and forecasting using data and statistical models. Statistics is applicable to a wide variety of academic disciplines, including natural and social sciences, government and business. Statistical consultants are available to provide help for organisations and companies without direct access to expertise relevant to their particular problems. Descriptive statistics can be used to summarise or describe a collection of data which is useful in research when communicating the results of experiments. Inferential statistics in a way accounts for randomness and uncertainty in the observations. This data is used to draw inferences about the process or population being studied. Inference is a vital element of scientific advance, since it provides a prediction (based in data) for where a theory logically leads. These predictions are part of the scientific method. If the inference holds true, then the descriptive statistics of the new data increases the soundness of that hypothesis. Descriptive statistics and inferential statistics together comprise applied statistics.

Statistics is closely related to probability theory, with which it is often grouped. The difference is roughly that in probability theory, one starts from the given parameters of a total population to deduce probabilities pertaining to samples, but statistical inference moves in the opposite direction, inductive inference from samples to the parameters of a larger or total population.

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3 Application of Statistics

In applying statistics to a scientific, industrial or societal problem, it is necessary to begin with a population or process to be studied. Populations can be diverse topics such as 'all persons living in a country' or 'every atom composing a crystal'. A population can also be composed of observations of a process at various times, with the data from each observation serving as a different member of the overall group. Data collected about this kind of 'population' constitutes what is called a time series.

For practical reasons, a chosen subset of the population called a sample is studied—as opposed to compiling data about the entire group (an operation called census). Once a sample that is representative of the population is determined, data are collected for the sample members in an observational or experimental setting. These data can then be subjected to statistical analysis, serving two related purposes: description and inference.

Descriptive statistics summarise the population data by describing what was observed in the sample numerically or graphically. Numerical descriptors include mean and standard deviation for continuous data types (like heights or weights), while frequency and percentage are more useful in terms of describing categorical data (like race).

Inferential statistics uses patterns in the sample data to draw inferences about the population represented, accounting for randomness. These inferences may take the form of answering yes/no questions about the data, or one can use it for hypothesis testing, estimating numerical characteristics of the data, describing associations within the data, that is, one can find out correlation, and modelling relationships within the data. One example is using regression analysis. Inference can extend to forecasting, prediction and estimation of unobserved values either in or associated with the population being studied. It can also include extrapolation and interpolation of time series or spatial data and can also include data mining (Leo Breiman 2001).

The concept of correlation is particularly noteworthy for the potential confusion it can cause. Statistical analysis of a data set often reveals that two variables (properties) of the population under consideration tend to vary together, as if they were connected. For example, a study of annual income that also looks at age of death might find that poor people tend to have shorter lives than affluent people. The two variables are said to be correlated. However, they may or may not be the cause of one another. The correlation phenomena could be caused by a third, previously unconsidered phenomenon, called a lurking variable or confounding variable. For this reason, there is no way to immediately infer the existence of a causal relationship between the two variables. For a sample to be used as a guide to an entire population, it is important that it is truly a representative of that overall population. Representative sampling assures that the inferences and conclusions can be safely extended from the sample to the population as a whole. A major problem lies in determining the extent to which the sample chosen is actually representative. Statistics offers methods to estimate and correct for any random trending within the sample and data collection procedures. There are also methods of experimental design for experiments that can lessen these issues at the outset of a study, strengthening its capability to discern truths about the population. Statisticians describe stronger methods as more 'robust'.

Randomness is studied using the mathematical discipline of probability theory. Probability is used in 'mathematical statistics' to study the sampling distributions of sample statistics, and in more general terms, the properties of statistical procedures can also be studied. The use of any statistical method is valid when the system or population under consideration satisfies the assumptions of the method.

Misuse of statistics can produce subtle but serious errors in description and interpretation—subtle in the sense that even experienced professionals make such errors and serious in the sense that they can lead to devastating decision errors. For instance, social policy, medical

practice and the reliability of structures like bridges all rely on the proper use of statistics.

Even when statistical techniques are correctly applied, then also the results are also very difficult to interpret for those people who are not experts in this field. The statistical significance of a trend in the data—which measures the extent to which a trend could be caused by random variation in the sample—may or may not agree with an intuitive sense of its significance. Statistical literacy is the set of basic statistical skills (and scepticism) that people need to deal with information in their day-to-day lives.

4 Statistical Methods

4.1 Experimental and Observational Studies

A common goal for a statistical research project is to investigate causality and in particular to draw a conclusion on the effect of changes in the values of predictors or independent variables on dependent variables or response. There are two major types of causal statistical studies: experimental studies and observational studies. In both types of studies, the effect of differences of an independent variable (or variables) on the behaviour of the dependent variable are observed. The difference between the two types lies in how the study is actually conducted. Each can be very effective. An experimental study involves taking measurements of the system under study, manipulating the system and then taking additional measurements using the same procedure to determine if the manipulation has modified the values of the measurements. In contrast, an observational study does not involve experimental manipulation. Instead, data are gathered, and correlations between predictors and response are investigated.

4.1.1 Experiments

The basic steps of a statistical experiment are:

1. Planning the research, including finding the number of replicates of the study, using the following information: preliminary estimates regarding the size of treatment effects, alternative hypotheses and the estimated experimental variability. Consideration of the selection of experimental subjects and the ethics of research is necessary. Statisticians recommend that experiments compare (at least) one new treatment with a standard treatment or control, to allow an unbiased estimate of the difference in treatment effects.
2. Design of experiments, using blocking to reduce the influence of confounding variables, and randomised assignment of treatments to subjects to allow unbiased estimates of treatment effects and experimental error. At this stage, the experimenters and statisticians write the experimental protocol that shall guide the performance of the experiment and that specifies the primary analysis of the experimental data:
   (a) Performing the experiment following the experimental protocol and analysing the data following the experimental protocol
   (b) Further examining the data set in secondary analyses, to suggest new hypotheses for future study
   (c) Documenting and presenting the results of the study

Experiments on human behaviour have special concerns. The famous Hawthorne study examined changes to the working environment at the Hawthorne plant of the Western Electric Company. The researchers were interested in determining whether increased illumination would increase the productivity of the assembly line workers. The researchers first measured the productivity in the plant, then modified the illumination in an area of the plant and checked if the changes in illumination affected productivity. It turned out that productivity improved under the experimental conditions. However, the study is heavily criticised today for errors in experimental procedures, specifically for the lack of a control, group and blindness.

4.1.2 Observational Study

An example of an observational study is one that explores the correlation between smoking and

lung cancer. This type of study typically uses a survey to collect observations about the area of interest and then performs statistical analysis. In this case, the researchers would collect observations of both smokers and non-smokers, perhaps through a case-control study, and then look for the number of cases of lung cancer in each group.

4.2 Levels of Measurement

There are four main levels of measurement used in statistics: nominal, ordinal, interval and ratio (Thompson 2006). Each of these has different degrees of usefulness in statistical research. Ratio measurements have both a meaningful zero value and the distances between different measurements defined. They provide the greatest flexibility in statistical methods that can be used for analysing the data. Interval measurements have meaningful distances between measurements defined, but the zero value is arbitrary. For example in the case with longitude and temperature measurements in Celsius or Fahrenheit. Ordinal measurements have imprecise differences between consecutive values but have a meaningful order to those values. Nominal measurements have no meaningful rank order among values.

Since variables conforming only to nominal or ordinal measurements cannot be reasonably measured numerically that is why sometimes they are grouped together as categorical variables, ratio and interval measurements are grouped together as quantitative or continuous variables due to their numerical nature.

5 Key Terms Used in Statistics

5.1 Null Hypothesis

Interpretation of statistical information can often involve the development of a null hypothesis in that the assumption is that whatever is proposed as a cause has no effect on the variable being measured. The best illustration for a novice is the predicament encountered by a jury trial. The null hypothesis, H_0, asserts that the defendant is innocent, whereas the alternative hypothesis, H_1, asserts that the defendant is guilty. The indictment comes because of suspicion of the guilt. The H_0 (status quo) stands in opposition to H_1 and is maintained unless H_1 is supported by evidence 'beyond a reasonable doubt'. However, 'failure to reject H_0' in this case does not imply innocence, but merely that the evidence was insufficient to convict. So the jury does not necessarily *accept* H_0 but *fails to reject* H_0. While one cannot 'prove' a null hypothesis, one can test how close it is to being true with a power test, which tests for type II errors.

5.2 Error

Working from a null hypothesis, two basic forms of error are recognised:

- Type I errors where the null hypothesis is falsely rejected giving a 'false positive'
- Type II errors where the null hypothesis fails to be rejected and an actual difference between populations is missed

Error also refers to the extent to which individual observations in a sample differ from a central value, such as the sample or population mean. Many statistical methods seek to minimise the mean-squared error, and these are called 'methods of least squares'.

Measurement processes that generate statistical data are also subject to error. Many of these errors are classified as random (noise) or systematic (bias), but other important types of errors (e.g. blunder, when an analyst reports incorrect units) can also be important.

5.3 Interval Estimation

Most studies are for only part of a sample population, and so the results are not fully representative of the whole population. Any estimates obtained from the sample only approximate the population value. Confidence intervals allow statisticians to express how closely the sample

estimate matches the true value in the whole population. Often they are expressed as 95% confidence intervals. Formally, a 95% confidence interval for a value is a range where, if the sampling and analysis were repeated under the same conditions (yielding a different dataset), the interval would include the true (population) value 95% of the time. This does not imply that the probability is the true value in the confidence interval, that is, 95%. From the frequentist perspective, such a claim does not even make sense, as the true value is not a random variable. Either the true value is or is not within the given interval. However, it is true that, before any data are sampled and given a plan for how the confidence interval will be constructed, the probability is 95% that the yet-to-be-calculated interval will cover the true value: At this point, the limits of the interval are yet-to-be-observed random variables. One approach that does yield an interval that can be interpreted as having a given probability of containing the true value is to use a credible interval from Bayesian statistics: This approach depends on a different way of interpreting what is meant by 'probability', that is, as a Bayesian probability.

6 Significance

Statistics rarely gives a simple yes/no type answer to the question asked. Interpretation often comes down to the level of statistical significance applied to the numbers and often refers to the probability of a value accurately rejecting the null hypothesis (sometimes referred to as the *p* value).

Referring to statistical significance does not necessarily mean that the overall result is significant in real world terms. For example, in a large study of a drug, it may be shown that the drug has a statistically significant but very small beneficial effect, such that the drug will be unlikely to help the patient in a noticeable way.

Examples. Some well-known statistical tests and procedures for reporting of data are:

- Analysis of variance (ANOVA)
- Chi-square test
- Correlation
- Factor analysis
- Mann–Whitney U
- Mean square weighted deviation (MSWD)
- Pearson product-moment correlation coefficient
- Regression analysis
- Spearman's rank correlation coefficient
- Student's *t* test
- Time-series analysis
- LC_{50} Bioassay test

7 Specialised Disciplines

Statistical techniques are used in a wide range of types of scientific and social research, including biostatistics, computational biology, computational sociology, network biology, social science, sociology and social research. Some fields of inquiry use applied statistics so extensively that they have specialised terminology. These disciplines include:

- Actuarial science
- Applied information economics
- Biostatistics
- Business statistics
- Chemometrics (for analysis of data from chemistry)
- Data mining (applying statistics and pattern recognition to discover knowledge from data)
- Demography
- Econometrics
- Energy statistics
- Engineering statistics
- Epidemiology
- Geography and geographic information systems, specifically in spatial analysis
- Image processing
- Psychological statistics
- Reliability engineering
- Social statistics

In addition, there are particular types of statistical analysis that have also developed their own specialised terminology and methodology:

- Bootstrap and jackknife resampling
- Multivariate statistics
- Statistical classification
- Statistical surveys
- Structured data analysis (statistics)
- Structural equation modelling
- Survival analysis
- Statistics in various sports, particularly baseball and cricket

Statistics form a key basis tool in business and manufacturing as well. It is used to understand measurement systems variability, control processes (as in statistical process control or SPC), for summarising data and to make data-driven decisions. In these roles, it is a key tool and perhaps the only reliable tool.

8 Statistical Computing

The rapid and sustained increases in computing power starting from the second half of the twentieth century have had a substantial impact on the practice of statistical science. Early statistical models were almost always from the class of linear models, but powerful computers, coupled with suitable numerical algorithms, caused an increased interest in nonlinear models as well as the creation of new types, such as generalised linear models and multilevel models.

Increased computing power has also led to the growing popularity of computationally intensive methods based on resampling, such as permutation tests and the bootstrap, while techniques such as Gibbs sampling have made use of Bayesian models more feasible. The computer revolution has implications for the future of statistics with new emphasis on 'experimental' and 'empirical' statistics. A large number of both general and special purpose statistical software are now available.

There is a general perception that statistical knowledge is all-too-frequently intentionally misused by finding ways to interpret only the data that are favourable to the presenter. If various studies appear to contradict one another, then the public may come to distrust such studies. For example, one study may suggest that a given diet or activity raises blood pressure, while another may suggest that it lowers blood pressure. The discrepancy can arise from subtle variations in experimental design, such as differences in the patient groups or research protocols, which are not easily understood by the nonexpert. By choosing (or rejecting or modifying) a certain sample, results can be manipulated. Such manipulations need not be malicious or devious; they can arise from unintentional biases of the researcher. The graphs used to summarise data can also be misleading. Deeper criticisms come from the fact that the hypothesis is a testing approach, widely used and in many cases required by law or regulation, forces one hypothesis (the null hypothesis) to be 'favoured' and can also seem to exaggerate the importance of minor differences in large studies. A difference that is highly statistically significant can still be of no practical significance. One response is by giving a greater emphasis on the p value than simply reporting whether a hypothesis is rejected at the given level of significance. The p value, however, does not indicate the size of the effect. Another increasingly common approach is to report confidence intervals. Although these are produced from the same calculations as those of hypothesis tests or p values, they describe both the size of the effect and the uncertainty surrounding it.

9 Statistics Applied to Mathematics or the Arts

Traditionally, statistics was concerned with drawing inferences using a semi-standardised methodology that was 'required learning' in most sciences. This has changed with use of fields as a degree requirement and is now viewed enthusiastically. Initially derided by some mathematical purists, it is now considered essential methodology in certain areas:

- In number theory, scatter plots of data generated by a distribution function may be

transformed with familiar tools used in statistics to reveal underlying patterns, which may then lead to hypotheses.

- Methods of statistics including predictive methods in forecasting are combined with chaos theory and fractal geometry to create video works that are considered to have great beauty.
- The process art of Jackson Pollock relied on artistic experiments, whereby underlying distributions in nature were artistically revealed. With the advent of computers, methods of statistics were applied to formalise such distribution-driven natural processes, in order to make and analyse moving video art.
- Methods of statistics may be used predicatively in performance art, as in a card trick based on a Markov process that only works some of the time, the occasion of which can be predicted using statistical methodology.
- Statistics can be used to predicatively create art, as in the statistical or stochastic music invented by Iannis Xenakis, where the music is performance-specific. Though this type of artistry does not always come out as expected, it does behave in ways that are predictable and tuneable using statistics.

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General Principles of Toxicity and Its Application

10

1 Introduction

A particular test should be widely accepted by scientific community and be able to predict the effects of wide range of chemicals on different organisms. Toxicity testing methods have been designed from time to time using different procedures and accordingly are designated as

1. Ecotaxonomic methods (single indicator species; multispecies or community and ecosystem studies)
2. Acute toxicity tests (mostly producing lethal effects)
3. Chronic and subchronic toxicity tests (producing lethal or sublethal effects and include entire reproductive cycle, early stages of life cycle, bioaccumulation tests, biochemical and physiological observations, behavioural responses, histopathological examinations)
4. Special methods

Various methods are:

1. Acute toxicity (single)
   (a) LD50 determination (1–2-week observation)
      (i) Two species (one non-rodent)
      (ii) Two routes of administration
   (b) Irritation studies
      (i) Dermal (rabbit)
      (ii) Eye irritation (rabbit)
2. Subacute toxicity
   (a) Duration 90 days
   (b) Two species (usually rat and dog)
   (c) Three dose species
   (d) Route of administration according to intended route of exposure
3. Chronic toxicity
   (a) Duration 2 years
   (b) Species (preferably two, one non-rodent species)
   (c) Three dose levels
   (d) Route of administration according to intended route of exposure
4. Short-term testing
   (a) Metabolism
   (b) Neurotoxicity
   (c) Reproduction and teratogenicity (at least one species)

2 Acute Toxicity

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 h). In acute toxicity, the adverse effects should occur within 14 days of the administration of the substance. Acute toxicity is distinguished from chronic toxicity, which describes the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years) (The MSDS HyperGlossary: Acute toxicity 2006).

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It is widely considered unethical to use humans as test subjects for acute (or chronic) toxicity research. However, some information can be gained from investigating accidental human exposures (e.g. factory accidents). Otherwise, most acute toxicity data comes from animal testing or, more recently, *in vitro* testing methods and inference from data on similar substances (Nic et al. 2006; Walum 1998).

2.1 Measures of Acute Toxicity

2.1.1 Regulatory Values

Limits for short-term exposure, such as STELs or CVs, are defined only if there is a particular acute toxicity associated with a substance.

- Short-term exposure limit, STEL; threshold limit value-short-term exposure limit, TLV-STEL
- Ceiling value, CV; threshold limit value-ceiling, TLV-C

2.1.2 Experimental Values

- No observed adverse effect level, NOAEL
- Lowest observed adverse effect level, LOAEL
- Maximum tolerable concentration, MTC, LC_0; maximum tolerable dose, MTD, LD_0
- Minimum lethal concentration, LC_{min}; mimimum lethal dose, LD_{min}
- Median lethal concentration, LC_{50}; median lethal dose, LD_{50}; median lethal time, LT_{50}
- Absolute lethal concentration, LC_{100}; absolute lethal dose, LD_{100}

2.1.3 Short-Term Testing

Acute toxicity or acute effects tests are rapid (2–4 days) procedures used to measure the concentration that will affect the test organisms.

Data from these tests can be used to:

1. Screen for toxicity (determine if the compound is toxic).
2. Rank toxicity to identify the best ingredients to continue investigating for use in a product.
3. Assess the potential for effects in the environment. In some cases, one group of organisms will be more sensitive to a compound than another group. For example, insecticides are usually more toxic to invertebrates than to fish or algae. When one starts a toxicity test programme, one may not know which group will be most sensitive to the new compound. So one usually tests at least one plant, one invertebrate and one fish species. It is important that all three groups of organisms are tested because all are important in the environment, and effects on a plant may not tell us anything about effects on an animal and vice versa.

Species typically used in acute toxicity tests include the following:

1. Lethality is the most common end point for invertebrates and fish, while growth of a population of cells is used to understand effects on algae.
2. The process we use to protect all these different species is called environmental risk assessment. When acute toxicity data does not provide enough information to decide if the compound is safe or not, chronic toxicity tests are carried out.

2.1.4 General Principles of Acute Toxicity Tests

The objective of acute toxicity test is to determine the concentration of a test material (e.g. chemical or effluent) or the level of an agent (e.g. temperature or pH) that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled conditions. Although toxicity tests with aquatic organisms can be conducted by administering the material directly by injection or incorporating it into food, most tests are conducted by exposure groups of organisms to several treatments in which different concentrations of the material are mixed with water. Because death is easily detected deleterious response, the most common acute toxicity test is the acute lethality test. Experimentally, a 50% response is the most reproducible measure of a test material, and 96 h is the standard exposure time because it covers the period of acute lethal action. Therefore, the measurement of acute toxicity used with fish and macro invertebrates is 96-h median lethal concentration (96-h LC50).

2.2 Single Species (Indicator) Tests

Current test methods are designed predominately to examine the responses of few individuals within a species. The response of an ecosystem consisting of interacting species is assumed to be predictable from single species toxicity tests. Most single species test are conducted in laboratory. These tests can provide a great deal of information on the concentration of chemicals and duration of exposure that produces changes in mortality, growth, reproduction, pathology, behaviour, physiology and biochemistry of organisms within species.

Cause and effect relationships can be established from single species tests because of degree of control over laboratory conditions. These tests are easy to conduct, and many are standardised and can be replicated. Current single species tests are conducted with individual species that are considered representative of broad classes of organisms so that the results provide information of toxicity of specific chemicals in different type of organisms under given conditions. Test species included are algae, crustaceans, fish etc. in aquatic systems. Single species laboratory tests cannot account for the adaptive ability of natural populations of organisms. Effects observed in laboratory tests thus appear more severe than those seen in field. Even then single species tests have yielded results that are well correlated with the observed ecological effects of chemicals.

2.3 Multispecies Tests (Community Structure)

These may be conducted in laboratory. These studies involve laboratory microcosms or model ecosystems. Laboratory microcosms are small-scale enclosures containing samples from natural ecosystem. Their advantage is that effects beyond the level of single species can be identified, providing information more directly related to ecological consequences of chemicals. In principle, if conditions are uniform, these tests should be easy to standardise and replicate. Since environmental influences are controlled, cause and effect relationship are more easily analysed than in natural systems. However, microcosms are oversimplifications of natural ecosystems. An excellent example of totally defined, material closed microcosm is performed test with 24 replicates using a total of 10 species of algae, 5 species of animals and unknown number of bacteria.

2.4 Acute Toxicity Tests

Acute toxicity tests have been conducted with industrial effluents for more than 40 years. Acute toxicity tests can be defined as the severe effects suffered by organisms from short-term exposure to toxic chemicals. These tests are designed to determine the dose or concentration of a particular test, chemical or effluent or the level of an agent that will produce a specific response on a group of test organisms during short-term exposure under controlled conditions. Since death is easily detected response, the most common acute toxicity test is the acute lethality test. The other two tests are irritation studies and potentiation or sensitising agents.

2.5 Lethality (LC50 or LD50)

This is one of the common ways to express acute toxicity. LC50 is statistical estimate of dose necessary to kill 50% of a large population of test species under stated conditions. Experimentally this is achieved by administering a chemical at graded doses to a group of organisms and then observing the resultant mortalities in a set time period, 96 h and so on. Among animals, rats, mice, rabbits, guinea pigs and hamsters are the test species. The measure of acute toxicity most frequently used with fish and microinvertebrates is the 96-h median lethal concentration. However, because death is not easily determined for some invertebrates, an EC50 is estimated rather than LC50.The effect used for estimating the EC50 with invertebrates like daphnids and midge larvae is immobilisation, that is, lack of movement. For EC50 of crabs, crayfish and shrimp, effects used are immobilisation and loss of equilibrium, defined as inability to maintain normal pressure.

Among plants, algae and duckweed (Lemna spp.) have been used as test organisms.

Acute toxicity tests methods may be categorised according to length of exposure, test situation, criteria of effects and test organisms. The data used in this test are used to determine the NOEC (no observed effect concentration or no effect concentration), which is maximum concentration of the test material that produces no statistically significant harmful effect on test organisms as compared to controls in a specific test, and LOEC (lowest observed effect concentration) or MTC (minimum threshold concentration) which is the lowest concentration that has statistically significant deleterious effect on test organisms compared to control in a specific test. In the early times of acute toxicity tests, data were expressed as the median tolerance limit (TLm or TL50)—the test material concentration at which 50% of test organism survive for a specific exposure time (usually 24–96 h). This term is now replaced by median lethal concentration (EC50).

2.6 Skin and Eye Tests

These tests are conducted with some chemicals that act as primary irritants. Chemicals when applied to skin or mucous membrane lead to tissue alteration of various types. The tests used indicate the propensity of a compound to cause tissue irritation and damage. When applied to eye, the tissue response may be severe and may lead to other toxic manifestations such as corneal damage and blindness. If inhaled, it may destroy tissue in respiratory tract. These tests are generally conducted in rabbits and guinea pigs, leading to damage of mucous membrane. An awareness of the primary tissue response for a specific chemical could alert individuals to handle the chemical with proper care.

2.7 Potentiation

The term potentiation or sensitisation means that when two chemicals are acting on same or different organs or systems, one chemical is made more effective in the presence of other chemical. This potential sensitising effect of chemical agents could become a major problem to toxicologists. The primary irritant elicits its effects immediately on its application. The sensitising agent may show no primary irritation but when applied or injected may combine with a protein molecule and acts as an antigenic agent. This in turn produces antibodies. If the compound is applied again or injected, an antigen antibody reaction may occur, producing either minor or serious allergic conditions which could lead to death or crippling illness. The tests are generally conducted on guinea pig, where the animal is first introduced to the chemical and then challenged by additional doses of the compound. Tissue response develops at the site of injection or elsewhere.

3 Subacute Toxicity Tests

3.1 Subacute Toxicity

Subacute toxicity study aims to find out toxic effect of drug on repeated exposure and also provide valuable information, that is, delayed effect which may result due to the cumulative effect of the chemicals on the tissues or other biochemical mechanisms. This study also helps in establishing the level of the safe usage of a compound.

The following routes are commonly used:

- Oral
- Dermal
- Intravenous
- Intraperitoneal
- Other protocol specified route

The period of exposure may vary from 14 to 90 days. One of the major objectives of subacute toxicity is to establish a dose which is classified as a no effect dose or that dose that which if exceeded can be considered harmful to man. For radiation or carcinogenic agents, a no dose effect perhaps is relevant, since the smallest dose will have a harmful effect. With very low doses of radiation or chemical carcinogens, the biological damage is not evident until fairly a prolonged period of exposure has occurred.

4 Chronic Toxicity Tests

A chronic toxicity test can indicate the concentrations of a chemical that will interfere with normal growth, development and attainment of reproductive potential of aquatic organisms. Generally, concentrations that produce chronic effects are lower than those that produce more readily observable acute effects such as mortality. Therefore, chronic toxicity tests can provide a more sensitive measure of chemical toxicity than acute toxicity tests. Three categories of tests are commonly used to predict the chronic and subchronic effects of toxic chemicals on organisms: (1) life cycle toxicity tests, (2) sensitive early stages tests and (3) functional tests.

4.1 Full Life Cycle Tests

These are typical chronic tests. These tests measure the effects of chronic exposure to a chemical on reproduction, growth, survival and other parameters over one or more generations of a population of test organisms. Groups of test organisms are exposed to a series of concentrations of test chemical. Chronic or full life cycle toxicity test is an important tool for understanding and evaluating the potential hazard of toxicants to organisms. A chronic toxicity test can indicate the concentrations of a chemical that will interfere with normal growth, development and attainment of reproductive potential of organism. The concentrations that produce chronic effects are lower than those producing more readily observable acute effects such as mortality. Therefore, chronic toxicity tests could provide a more sensitive measure of chemical toxicity than acute toxicity tests. The duration of test varies with the species tested. It is approximately 21 days for water flea, and it can be 275–300 days for the fathead minnow. In fish, each of the several groups of individuals of one species is exposed to a different concentration of a toxicant throughout a life cycle to study the effect of toxicant on survival, growth and reproduction of the species.

4.1.1 Maximum Acceptable Toxicant Concentration (MATC)

This is the threshold data that produces statistically significant deleterious effect. The MATC is hypothetical concentration and is in a range bounded at the lower end by the highest concentration in the chronic test that produced no effect (NOEC, no observed effect concentration) and at the higher end by the lowest concentration tested that produced statistically significant effect (LOEC, lowest observed effect concentration). Therefore, MATC can be represented as NOEC $<$ MATC $<$ LOEC, for example, 0.05 ppm $<$ MATC $<$ 1.0 ppm.

4.1.2 Partial Life Cycle Toxicity Tests

Each of several groups of individuals of one species is exposed to a different concentration of toxicant throughout part of life cycle which includes life stages observed to be especially sensitive to chemical exposure. Such tests on fish like brook trout and blue gills require more than 1 year. Test can be completed in less than 15 months, and all the major life stages are exposed to toxicant.

4.2 Most Sensitive Early Life Stages Toxicity Tests

During life cycle tests in vertebrates and invertebrates, some developmental stages are more sensitive than other stages. The most sensitive stages are used to predict chronic toxicity of chemicals to organisms. Most vertebrates' life cycle toxicity tests have been conducted in fish. In early life stages test, most sensitive life stages thus measure the effect of chronic exposure on survival and growth of the most sensitive life stages, as eggs and larva of fish. Relatively a short exposure of the embryo-larval and early juvenile stages of fish to a toxicant can be used to estimate MATC without a complete life cycle test. These tests thus include continuous exposure of the ELS (e.g. egg, embryo, larva and fry) of aquatic organisms to various concentrations of a chemical for 1–2 months depending upon species. Various developmental periods and developmental phases of a typical teleost, essential in ELS toxicity tests, are as follows:

4.2.1 Embryonic Period

It begins with fertilisation and ends at hatching. This period is divided into two phases.

(a) *Cleavage Phase*. It involves the first interval of development within the egg membranes, from the beginning of the development to organogenesis. Most ELS toxicity tests begin early in this phase, although some begin at fertilisation.
(b) *Embryonic Phase*. It involves the interval of intense organogenesis within the egg membranes and continues until hatching is completed.

4.2.2 Larval Period

It begins with hatching of the egg and lasts until the disappearance of the last vestige of the embryonic median fin fold and the appearance of a full complement of fin rays and spines. This period is divided into three phases:

(a) *Protolarva*. This is the larval phase with no dorsal and/or caudal spines or rays apparent. The only median fin elements present are the dorsal and ventral fin folds.
(b) *Mesolarva*. This is the larval phase where at least one of the principal rays is apparent in the median fin, or if all fin rays are present in the median fin and adult has pelvic fins, the pelvic buds or fins are apparent.
(c) *Metalarva*. This is a larval phase where all principal median fin rays are present. If the adult has pelvic fins, the pelvic buds or fins are apparent. Salmonoids do not go through the three phases. Their larval period is represented by the yolk sac or alevin period which begins with hatching and ends after complete absorption of the yolk, with the juvenile.

4.2.3 Juvenile Period

It begins with when all the fins are fully differentiated and median fold is apparent. The body is entirely scaled. The juvenile is in fact a miniature adult in appearance. Most ELS toxicity tests with fish end shortly after metamorphosis from larval to juvenile period.

4.2.4 Adult Period

It begins with the first maturation of gamete and is accompanied by secondary sexual characteristic and spawning behaviour.

4.2.5 Senescent Period

It is old age, accompanied by slow to no growth and few to no gametes.

4.3 Functional Tests

These tests measure the chronic toxicity of chemicals through various physiological functions or functional responses of organisms. Functional responses include physiological and behavioural responses as affected due to exposure of sublethal concentrations of toxic chemicals. Different organisms when exposed to toxicants change blood chemistry, histology, swimming performance, avoidance, respiration, enzyme activities, sensory perception and disease resistance.

Functional tests are also used in studies on bioaccumulation and biotransformation process. Many of the functional responses are sensitive indicators of sublethal toxic effects.

4.3.1 Behavioural Responses

Behaviour is the observable, recordable or measureable activities of a living animal. It is everything an animal does, including all of its integrated movements. Animal behavioural patterns are highly adaptive to environmental variables of physical, chemical or biological nature. Chemical agents may also be behaviourally toxic in organisms. Behavioural toxicity occurs when the introduction of a chemical induces a behavioural change that exceeds the normal range of variability. Most behavioural toxicity tests have been made on fish and other aquatic organisms.

4.3.2 Enzyme Activities

Some enzyme activities can be used as a criteria of measuring toxicity of a chemical. These functional responses can be used mainly in studies on bioaccumulation, biotransformation and biomonitoring of chemical toxicants.

Lysosomal Stability. Lysosomes are organelles responsible for the breakdown of exogenous and endogenous substrates in animal cells. A good regulation system of permeability of lysosome membrane is needed to prevent catalytic enzymes from entering the cytoplasm. Under conditions of low chemical stress, only small molecules can penetrate the membrane into the lysosome; under serious stress, larger molecules may pass the membrane. A number of xenobiotics may accumulate in the lysosome as a result of low biodegradability.

Mixed Function Oxidase (MFO) Activity (Microsomal Oxidations). The MFO system is membrane-bound non-phosphorylating electron transport complex that normally forms part of endoplasmic reticulum. The system metabolises natural substrates (steroids, fatty acids) as well as xenobiotics, including pesticides. One of the MFO components is the iron-containing cytochrome P450. The compound neotetrazolium (NTR) is also able to accept electrons from cytochrome P450 in this manner. Thus, NTR reduction may be used as a measure for MFO activity in the hepatopancreas tubuli as well as in blood cells. The quantitative determination of the NTR-reduction products can be carried out with a microdensiometer.

Metallothionein Induction. Metallothioneins are specific proteins without any specific functions, in which cysteine is an important constituent. Metal ions are easily bound by cysteine, counteracting toxic effects. Induction of metallothionein synthesis in animals as mussels takes place in various tissues as gills, mantle and especially hepatopancreas after exposure to Cd, Cu and Zn.

4.3.3 Histopathological Examinations

Histopathology, the study of the structure of abnormal tissue, has important applications in toxicology. Most histological and histopathological studies are made on mammals. There are routine diagnostic tests to evaluate abnormal tissue changes resulting from exposure of an organism to the toxicant.

4.4 Other Toxicity Tests

There are several other toxicity tests used in special studies on evaluating the effect of toxic chemical on organisms by taking suitable parameters.

4.4.1 Reproduction Toxicity and Teratogenicity

Reproduction toxicity is interference by an agent with the reproductive processes in one or more ways. The toxicant may lead to malformation in children, reduce chance of conception, may be embryocidal, cause stillbirth or death of the progeny. Since the incidence of malformation from thalidomide in children, reproduction toxicity and teratogenic studies became part of safety evaluation of new drugs and chemicals.

4.4.2 Teratology

Teratology is the study of effects of intrinsic factors related to permanent structural and functional abnormalities during the period of embryo development. Teratogenicity is the experimental induction of developmental abnormalities. Teratogens may be drugs, food additives or other chemicals, physical agents, infectious microbes, hormonal and metabolic agents, nutritional or environmental factors.

4.4.3 Carcinogenicity

Many drugs and chemicals are known for slowly developing, cumulative and irreversible organ damage. Carcinogenic effects are the most common of these effects. Chemicals are taken orally, parenterally or by inhalation. Chemicals may also be applied to the skin. The drugs or chemicals are tested *in vitro* and *in vivo*.

4.4.4 Mutagenicity

Animal experiments or bacterial test systems are used to identify strong mutagens. Several *in vitro* and *in vivo* test systems have been evaluated, but with low doses, the mutagenic effects are often missed. Due to development and use of *in vitro* bacterial bioassays, much progress has been made in this area. Several other systems are being evaluated for rapid screening of a large number of materials over a wide range of concentrations.

4.5 Bioassay in Toxicity Testing

Bioassay is frequently used in toxicity tests, chiefly aquatic toxicity. A bioassay is a test to evaluate the relative potency of a chemical by comparing its effect on a living organism with that of a standard preparation. A bioassay is performed to determine the strength of the chemical from the degree of response produced by a specific level of stimulus. Bioassays are frequently used in the pharmaceutical industry to evaluate the potency of vitamins and other pharmacologically active compounds. Bioassay is thus a test in which organisms are used to detect the presence or the effects of physicochemical factors in the environment. The test involves exposure of animal to the toxicants for a definite period in the static laboratory environment and observing survival or other symptoms during a period of 96 h or so.

5 Good Laboratory Practices (GLP)

GLP is concerned with the organisational process and the conditions under which laboratory studies are planned, performed, monitored, recorded and reported. The GLP requirements deals with all related aspects of testing, namely, personnel, facilities, equipment, laboratory operations, test chemicals, protocols, reports and disqualification of testing facilities.

6 Cumulative Toxicity

There is no consistent relationship between the acute and chronic toxicity because the course of death after a single high dose may be entirely different from that encountered after repeated administration of subacute doses. Species with biotransformation similar to that in average man is selected for test of cumulative toxicity. The duration of the toxic effects of drug or chemical varies with the rate of metabolic detoxification and elimination. If this is not complete or almost complete by 24 h then a second dose is given in multidose studies, the toxic effects of the first dose will be added in part to those of the second dose, producing cumulative toxicity.

The use of 100-day LD50 index for the cumulative toxicity evaluation of chemicals and drugs has been suggested. There are given daily doses with increasing fractions of the acute LD50. Percent mortalities from each daily dose are tabulated at weekly intervals. From these, LD1, LD50 and LD99 calculated at 100 days are referred to as LD50 (100 days). The 100-day LD50 index is calculated by expressing the LD50 (100 days) as percentage of the acute LD50 (1 dose), that is,

$$100\,\text{days LD50 index} = \frac{\text{LD50 (100-days)}}{\text{LD50 (1-days)}} \times 100$$

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Fate of Biotoxins in the Environment and Its Health Implications

11

1 Introduction

Biotoxins can be of animal or plant origin or from microorganisms. Toxicity is the function of dose and susceptibility of the host. There is always a safe limit for any toxin, and similarly every known substance can produce a toxic effect beyond a certain point. A biotoxin is a chemical produced by any living organism either for defence or offence and can effect and kill other living organisms. Poisonous animals are found in all groups including birds, that is, from *Alexandrium* to chordates including duckbilled platypus and short-tailed shrew. There are about 1,200 species of poisonous marine animals and 400 species of poisonous snakes. Creatures who have developed secretory glands or groups of cells for producing poison and deliver it during a bite or stinging act are called venomous, and those without specialised mechanisms but whose tissues are toxic are termed poisonous. There are two types of biotoxins: one is of animal origin and the other is of marine origin.

2 Biotoxins of Animal Origin

Venom is important for an animal's survival. Its role can be for:

1. Offence for capture and digestion of prey, for example, snakes
2. Defence against predators, offences as well as defences
3. Paralysis of prey before catching it, for example, black widow spider

2.1 Properties of Animal Toxins

Chemically, toxins are proteins of high or low molecular weight including polypeptides and enzymes (Russell 1996). They can also be amines, lipids, steroids, amino-polysaccharides, quinines, 5-hydroxytryptamine and glycosides. Generally, many of the venoms have high affinity for a particular tissue, causing more damage to that tissue. The toxic activity is named after the specific tissue that the venom targets, for example, neurotoxins, cardiotoxins, hemotoxins and mycotoxins, even though other tissues are affected with higher doses. The more important types of toxins produced by different organisms are described below:

2.2 Snake Venoms

These venoms contain at least 25 types of proteins with enzymatic activity (Lee 1979). Out of these, some are proteolytic enzymes; arginine ester hydrolase; thrombin-like enzymes; collagenase; hyaluronidase; phospholipase A, B, C; lactase dehydrogenase; phosphomonoesterase; phosphodiesterase; acetylcholinesterase; RNAse; DNAse; 5′-nucleotidase; and L-amino acid oxidase (Russell 1967, 1980, 1983). Venom contains inorganic substances/metals like Na,

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K, Ca, Mg, Zn, Fe, Co, Mn and Ni. Some of the proteases may be metalloproteins, and other constituents are lipids, biogenic amines or free amino acids (Elliot 1978; Habermehl 1981).

2.3 Polypeptides

They are low molecular weight nonenzymatic proteins grouped as neurotoxins since they have special affinity for the nervous system. Out of these 80 polypeptides have been isolated.

2.4 Lizard Venoms

They are not very toxin and contain serotonin, amine oxidase, phospholipase A, proteolytic and hyaluronidase activities.

2.5 Amphibian Toxins

Amphibian toxins are present in the cutaneous secretory glands. They act as defence against predators and prevent the growth of micro-organisms on the skin. Toad skin contains more than 300 steroidal alkaloids, some of which are batrachotoxins and samandarines. Biogenic amines like adrenaline, noradrenaline, dopamine and epinine and indolealkylamines such as bufotenin, bufotenidin and bufoviridine are also present. Frog skin contains zetekitoxins A, B and C, tetrodoxin, chiriguitoxin and batrachotoxin.

2.6 Scorpion Toxins

Out of 800 species of scorpions, almost 75% are medically important. The toxins cause local reaction as well as affect the cardiovascular and central nervous systems causing salivation, muscular paralysis, convulsions and respiratory depressions.

3 Toxins of Marine Origin

Marine toxins are some of the strongest toxins known (Van Dolah 2010). Chemically, they contain proteins of low and high molecular weight, lipids, amines, alkaloids, guanidine bases and mucopolysaccharides. In general marine fauna and flora can be toxic, and they cause several epidemics in the coastal areas. Some marine animals particularly fish, mussels and clams may not be themselves poisonous, but the toxins get biomagnified through the food chain as they may feed on toxic dinoflagellates, other diatoms and algae and become toxic to humans even though they themselves do not suffer. Marine mammals may be exposed to environmental stressors such as chemical pollutants, harmful algal biotoxins and emerging or resurging pathogens. Since many marine mammal species share the coastal environment with humans and consume the same food, they also may serve as effective sentinels for public health problems. Marine mammals are sentimental species for oceans and human health (Bossart 2011). Finally, marine mammals are charismatic megafauna that typically stimulate an exaggerated human behavioural response and are thus more likely to be observed. Seafood is high on the list of food transmitting disease. However, the food safety issues are highly focussed, and more than 80% of all seafood-borne outbreaks are related to biotoxins (ciguatoxin), scombrotoxin or the consumption of raw molluscan shellfish. It is pointed out that there are serious safety concerns related to the consumption of raw fish and shellfish due to the presence of biological (bacteria, virus, parasites) and chemical (biotoxins) hazards. These hazards are present in the fish and shellfish preharvest and are difficult or impossible to control by applying presently available preventive measures. In contrast, the hazards related to contamination, recontamination or survival of biological hazards during processing are well defined and can be controlled by applying good

manufacturing practice (GMP), good hygiene practice (GHP) and a well-designed HACCP programme. Similarly, to prevent the growth of pathogenic microorganisms during distribution and storage of the final products are—with a few exceptions—available. Proper application of well-known preservative parameters including temperature is able to control growth of most pathogens. When this is not always the case, for example, inhibition of *Listeria monocytogenes* in lightly preserved fish products, it is recommended to limit the stated shelf life of these products to a period of no growth for the pathogen of concern. There is good agreement between the trends shown in disease statistics, the hazard analysis and the qualitative risk assessment of the various fish products. It is recommended that consumers should be informed of the risk of eating raw seafood—particularly molluscan shellfish and certain freshwater fish.

Toxic marine organisms are distributed in all classes of animals, and some of the important ones are as follows:

3.1 Marine Organisms Containing Toxins

1. *Dinoflagellates*: They are best known causing paralytic shellfish poisoning and red tides. They are accumulated in the digestive glands of mussels and clams. Some examples are *Prorocentrum, Dinophysis, G.polyedra, Pyrodinium, Ostreopsis, Gonyaulux catenella, Gambierdiscus* and *Gymnodinium breve*.
2. *Porifera*: Many sponges are poisonous. Examples include *Tedania nigrescens* and *Neofibularia nolitangere*.
3. *Coelenterata* (hydroids, jellyfish, sea anemones, corals):
   Sea anemones, for example*, Anemonia sulcata*, *Actinia equina*
   Stinging jellyfish, for example*, Aurelia*, *Carybdea*
4. *Echinodermata* (starfish, sea urchins, sea cucumbers):
   Acanthaster planci (asteroid)
   Sea urchins: *Paracentrotus lividus, Tripneustes ventricosus, Centrechinus antillarum*
5. *Mollusca* (snails, bivalves, cephalopods):
   Neptuna conus, Murex ommastrephes, Octopus apollyon, Aplysia californica
6. *Fish*: 700 species of marine toxic fishes are known. Some of the more important ones are *Somniosus microcephalus, Mugil cephalus, Neomyxus chaptalli*

Many marine toxins are still not chemically characterised; examples of some that have been studied in detail are as follows:

Source	Toxins
A. Flora	
Dinoflagellates	Domoic acid, okadaic acid, anatoxin, brevetoxin, saxitoxin, neosaxitoxin, gonyautoxins (I–VIII), C3 and C4 toxins, ciguatoxin, maitotoxins, cyanoginosin, aphanorphine
Chondria armata	Domoic acid
B. Fauna	
1. Scorpion fish	Stonefish toxin
2. Japanese 'fugu' or puffer fish	Tetrodotoxin
Ocean sunfish, salamandrine	Maculotoxin
Frogs and octopus	Chiriguitoxin
3. Sea cucumber	Holotoxin
4. Sea sponges	Halitoxin

Relative toxicities of some marine flora and fauna (tested in mice)

Organism	Toxin	LD50 (per kg i. p.)
Palythoa mammilosa	Palytoxin	50–100 ng
Gambierdiscus toxicus	Maitotoxin	170 ng (MLD)
G.toxicus	Ciguatoxin	450 ng
Saxidomus giganteus	Saxitoxin	10 μg
Tapes semidecussata	Tetrodotoxin	8–20 μg
Laticauda semifasciata	Laticatoxin	130 μg
Ptychodiscus brevis	Brevetoxin	250 μg
Lophogorgia sp.	Lophotoxin	8 mg
Holothuria tubulosa	Holotoxin	5–15 mg iv
Lumbiconeresis heteropoda	Nereistoxin	33 mg iv
Haliclona viridis	Halitoxin	2.5 mg iv

4 Toxins of Plant Origin

Many plant toxins have been studied and are in use as medicines. However, if the recommended dosages are exceeded, they can become dangerous. The more important toxins act on different parts of the body. Many toxins can cause morbidity including allergy and mortality (Norton 1996; Dawson 1998).

4.1 Cardiovascular System

Several plants are toxic through inadvertent ingestion by humans or grazing by cattle (Gopalkrishnan and Tan 1992). The more important of these are the rhizomes of *Veratrum album*, *V. viride*, *V. californicum* and seeds of *Schoenocaulon officinale*. The specific toxic ingredients are the alkaloids veratridine, protoveratrine, veratramine and jervine. When ingested, they cause hypotension, bradycardia and muscular twitchings. They act on the sodium current in the heart resulting in the depolarisation of the membrane. Aconitine alkaloid from *Aconitum napellus* causes cardiac arrhythmia and repeated firing of nerves due to action on Na channels. *Taxus baccata* leaf intake has been reported to cause death due to cardiac or respiratory failure as it inhibits Ca^{++} current in the heart. Taxol derived from *T. brevifolia* has anticancer properties. Another plant, *Ryania speciosa*, causes muscle contraction and has insecticidal properties. The active alkaloid responsible for this activity is ryanodine.

Other well-known plants that have medicinal properties and are used clinically include *Digitalis purpurea* (foxglove) from which digitalis has been extracted, *Scilla maritima* (squill) from which scillaren has been isolated, *Convallaria majalis* (lily of valley) from which convallatoxin has been isolated, *Nerium oleander* (bay laurel) from which oleandrin and neroside have been isolated, *Thevetia peruviana* (yellow oleander) from which thevetin A and B have been isolated and *Strophanthus gratus* from which strophanthin G has been isolated. They all cause ataxia, tremors and convulsions in higher doses but are cardiotonic in smaller doses. *Lobelia inflata* was found to cause emesis and affects the cardiovascular system. The active ingredient causing this effect was found to be lobeline, which has high affinity for nicotinic cholinergic receptors. *Nicotiana tabacum* yields nicotine, an alkaloid in nature with central nervous system (CNS) stimulant and other cardiovascular effects produced by action on nicotinic receptors. *Phoradendron tomentosum* is a parasitic plant with toxic berries. Isolation of phoratoxin has been reported. It is a polypeptide with a molecular weight of about 13,000 and causes hypotension, bradycardia, negative inotrophic effect on the heart and vasoconstriction of skin and skeletal muscles. Another parasitic plant is *Viscum album* from which polypeptides viscotoxins have been isolated. They are five times more toxic than phoratoxins. A lectin viscumin has been isolated which is cytotoxic and used as anticancer agents. Among the fungi, *Claviceps purpurea*, a parasite of rye grain yields ergot. It is well known to cause vasoconstriction of the extremities leading to gangrene and also has a stimulant action on uterus. Another fungus, *Acremonium coenophialum*, grows symbiotically on the forage grass and tall fescue and produces ergot alkaloids and lysergic acid derivatives. Animals grazing on the grass are adversely affected.

4.2 Nervous System

Strychnine has been isolated from seeds of *Strychnos nux-vomica*. It is a heterocyclic alkaloid and causes convulsions in animals and humans. Cocaine has been isolated from *Erythroxylon coca* leaves. Chemically, it is benzoylmethylecgonine and causes central nervous system (CNS) stimulation. *Chondrodendron* yields curare, a well-known neuromuscular blocking agent, which can cause death in very small doses. *Anabaena flos-aquae* (blue-green alga) grows in ponds in summer and produces a neurotoxin called anatoxin-a which kills animals drinking affected water through a

neuromuscular blocking effect. *Delphinium barbeyi* has yielded an alkaloid called methyl lycaconitine which kills cattle like curare. It is used as a pharmacological tool since it blocks only nicotinic receptors. *Swainsona canescens, Astragalus lentiginosus* and *Oxytropis sericea* produce hyperexcitability and ataxia in cattle that graze on them due to the presence of an indolezidine alkaloid, swainsonine; *Digenea simplex* (red alga) yields kainic acid (cyclic amino acid), which causes depolarisation of crayfish and insect mussel, and *Mytilus edulis* and *Chondria armata* (seaweed) has also yielded a cyclic amino acid which is excitotoxic like glutamic acid and aspartic acid and causes neuronal death leading to headache, hemiparesis, confusion, seizures and amnesia.

Some mushrooms have a toxic effect on the CNS. *Amanita muscaria* has been found to contain a quaternary ammonium alkaloid, ibotenic acid, which is an agonist of the excitatory amino acid receptors causing depolarisation of the neurons in brain. *A. pantherina* also produces ibotenic acid as well as muscimol which causes CNS depression, ataxia, hallucinations, myoclonic twitchings and seizures. Plants like *Acacia willardiana* and *A. lemmoni* also produce an excitatory amino acid in their seeds called willardiine. It is an agonist of the kainate receptors. *Lathyrus sativus* and *L. sylvestris* produce diaminobutyric acid (DABA) and β-oxalyl diaminopropionic acid (BOAA) in their seeds. Both are excitatory neurotoxins and can lead to spastic muscle weakness and atrophy. They decrease bronchial and salivary secretions in low dose, but the large doses affect the CNS causing confusion, hallucinations and amnesia.

4.3 Gastrointestinal Tract (GIT)

A number of plants have been found to affect the GIT ranging from mild to severe depending on the dose. *Rhamnus purshiana* contains emodin and acts as a purgative. *Wisteria floribunda* seeds contain lectin, and a few seeds taken orally can cause headache, nausea, vomiting, diarrhoea and haematemesis. *Ricinus communis* seeds contain ricin I and II and can produce all GIT symptoms and icterus in fatal cases (Stewart et al. 2006).

4.4 Liver

The liver is the most important metabolic centre of the body. Many plants and fungi can damage it. Several nonedible mushrooms like *Amanita phalloides* and *A. ocreata* have phalloidin, and amatoxins are very toxic and can cause mortality. Among the fungi, *Fusarium moniliforme* grows on corn and can affect horses. They suffer from lethargy, ataxia, convulsions and death due to effect in brain and liver. In humans they may cause oesophageal cancer (Tu 1977).

Some of the more important toxins are briefly described below:

4.5 Okadaic Acid (OKA)

Produced by dinoflagellates and can cause toxicity at concentrations of 0.5–1.0 nM. It is a potent inhibitor of protein phosphatases PP1 and PP2A leading to significant increase in protein phosphorylation and alters the activity of Ca^{++}/calmodulin-dependent protein kinase II (CaM-K). It is tumour promoter and cellular functions such as smooth muscle contraction, fatty acid biosynthesis, protein synthesis, catecholamines and their secretions. Liquid chromatography (HPLC) was used to search for esters of DSP toxins in Portuguese bivalves. Hexane-soluble derivatives of okadaic acid (OA) and dinophysistoxin-2 (DTX-2) were found. Presumably, they are acyl derivatives, globally known as 'dinophysistoxin-3' (DTX-3). In certain instances DTX-3 content surpassed 50% of the total amount of DSP toxins. A human diarrhoeic poisoning (DSP) incident with Donax clams (*Donax trunculus*) harvested at Fuzeta (Algarve coast) was confirmed where the apolar (DTX-3 type) and other esters remaining in the polar aqueous methanol layer were implicated. The percentage of acyl esters of OA was always higher than those of DTX-2. An

enzymic mechanism for the conversion of OA and DTX-2 seems to be implicated in some kind of detoxification process because the percentage of esters increases with the toxin amount ingested by the bivalve, and there is some degree of selectivity as DTX-2 seems more difficult to acylate (Fig. 11.1). These findings pose a problem for the current assay methods used to detect DSP because mainly they are able to detect the parent toxins but not their esters. The current bioassay method used in Portugal includes a hexane washing step that prevents interference from free fatty acids. However, it cannot detect the presence of acyl derivatives because they are highly soluble in hexane (Vale et al. 1999).

4.6 Domoic Acid

Domoic acid (DA), the neurotoxin that causes amnesic shellfish poisoning (ASP), is a kainic acid analogue, heterocyclic amino acid associated with certain harmful algal blooms.

In 1958, domoic acid was originally isolated from the red alga called 'doumoi' or 'hanayanagi' (*Chondria armata*) in Japan. 'Doumoi' is used as an anthelmintic in Tokunoshima, Kagoshima. Domoic acid is also produced by some diatoms of the genus *Pseudo-nitzschia* and by some strains of the diatom species *Nitzschia navis-varingica*. *Pseudo-nitzschia multiseries* loses most of its ability to produce domoic acid when it is cultured axenically. However, domoic acid production recovers when bacteria from the original culture are reintroduced to axenic cultures, indicating a bacterial association with domoic acid production in this species. The increasing frequency and geographic extent of toxic algal blooms along populated coastlines is generally attributed to human activities. Considerable recent research has been carried out by the Marine Mammal Center and other scientific centres on the association of domoic acid-producing harmful algal blooms and neurological damage in marine mammals of the Pacific Ocean. Domoic acid can bioaccumulate in marine organisms such as shellfish, anchovies and sardines that feed on the phytoplankton known to produce this toxin. It can accumulate in high concentrations in the tissues of these plankton feeders when the toxic phytoplankton itself is high in concentration in the surrounding waters.

In mammals, including humans, domoic acid acts as a neurotoxin, causing short-term memory loss, brain damage and, in severe cases, death. DA-producing algal blooms are associated with the phenomenon of amnesic shellfish poisoning (ASP). In marine mammals, domoic acid typically causes seizures and tremors. In the brain, domoic acid especially damages the hippocampus and amygdaloid nucleus. It damages the neurons by activating AMPA and kainate receptors, causing an influx of calcium. Although calcium flowing into cells is a normal event, the uncontrolled increase of calcium causes the cell to degenerate. Because the hippocampus may be severely damaged, short-term memory loss occurs. It is a rare excitatory tricarboxylic amino acid. It is activated by dicarboxylic acid, aspartic acid and glutamic acid and is mainly neurotoxic in behaviour (Fig. 11.2).

4.7 Ciguatoxin (Fig. 11.3)

The ciguatoxins are a class of poisonous organic compounds found in some fish that causes ciguatera.

4.7.1 Toxic Effect

Ciguatoxin lowers the threshold for opening voltage-gated sodium channels in synapses of the nervous system. Opening sodium channel causes depolarisation, which could sequentially cause paralysis, heart contraction and changing the senses of hearing and cold. Because they do not cross the blood–brain barrier (BBB), ciguatoxins solely affect the peripheral nervous system (PNS). The major symptoms will develop within a few hours of toxin ingestion: vomiting; diarrhoea; numbness of extremities, mouth and lips; reversal of hot and cold sensation; and muscle and joint aches. The symptoms may last from days to weeks or even months depending on each individual situation. There is no antidote for ciguatoxin poisoning.

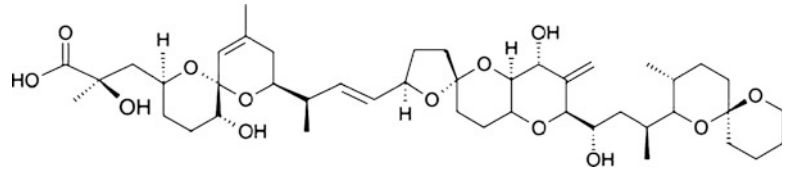

Fig. 11.1 Structure of okadaic acid

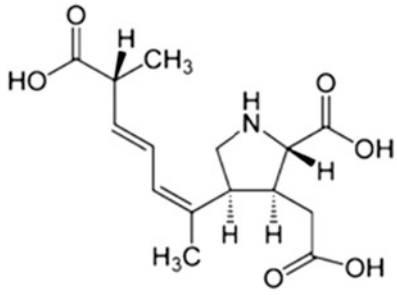

Fig. 11.2 Structure of domoic acid

4.7.2 Bioaccumulation Route

Ciguatoxin is produced by *Gambierdiscus toxicus*, a type of dinoflagellate, which are eaten by big coral reef fish, such as grouper, wrasse, triggerfish, lionfish and amberjack. Ciguatoxin usually accumulates in skin, head, viscera and roe of the fish. Ciguatoxin cannot be destroyed by cooking. Rapid testing for this toxin in fish marketed as food is not standard. It causes enteric illness followed by paraesthesia, rheumatic complaints, visual abnormality and bone and tooth pain and can be potentially lethal.

4.8 Scombrotoxin

Produced by bacterial action especially by *Proteus morganii* on unrefrigerated fish flesh. Fish consumption may be followed by a brief enteric phase followed by headache, pruritus, urticaria, bronchospasm and abdominal cramps due to absorption of histamine from gastrointestinal tract. Scombrotoxin is a foodborne toxin most often associated with the consumption of fish, particularly species belonging to the *Scombridae* and *Scomberesocidae* families (scombroid fish), such as mackerel and tuna. It can cause a mild, though sometimes distressing, form of foodborne intoxication (scombroid or scombrotoxic food poisoning) when ingested in sufficient quantities.

Scombrotoxic poisoning is also known as histamine poisoning, since histamine is considered to be the toxic component of scombrotoxin, although other compounds may be involved. Histamine ($C_5H_9N_3$) is a biogenic amine and can be produced during processing and/or storage in fish and certain other foods, usually by the action of spoilage bacteria.

4.8.1 Occurrence in Foods

Scombrotoxin is most often associated with scombroid fish, especially tuna, skipjack, bonito and mackerel, but other non-scombroid fish, such as sardines, herring, pilchards, marlin and mahi-mahi, have also been involved in outbreaks of illness. There are also reports that scombrotoxin could occur in salmon species. Generally, fast-swimming and migratory finfish species with red-coloured meat are more likely to develop high histamine levels than whitefish species. The toxin is not limited to fresh and frozen fish. It may be present in canned and cured fish products at high enough concentrations to cause illness. The concentration of histamine can vary considerably between different sampling sites in a single fish or between individual cans in a single lot. Levels of >3,000 ppm have been recorded in fish products implicated in outbreaks. Histamine can also be produced at levels toxic to humans by bacterial action in other foods, notably Swiss cheese.

4.8.2 Hazard Characterisation

Effects on Health

Scombrotoxic (histamine) poisoning is a chemical intoxication, in which symptoms typically develop rapidly (from 10 min to 2 h) after ingestion of food containing toxic histamine levels. The range of symptoms experienced is quite

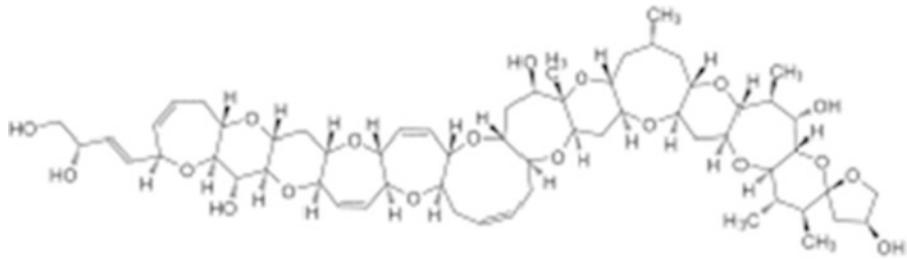

Fig. 11.3 Structure of ciguatoxin

wide, but may include an oral burning or tingling sensation, skin rash and localised inflammation, hypotension, headaches and flushing. In some cases vomiting and diarrhoea may develop, and elderly or sick individuals may require hospital treatment. The symptoms usually resolve themselves within 24 h. The evidence for histamine as the active toxin in scombrotoxic poisoning is strong, but the condition is very difficult to replicate in humans using pure histamine. It is possible that other biogenic amines in spoiled fish, such as putrescine and cadaverine, may act as potentiators for histamine toxicity, but the mechanism for this is not known.

For this reason the threshold toxic level for histamine remains uncertain. Individuals also vary in the severity of their response to histamine in fish. Analysis of outbreaks suggests that levels of histamine above 200 ppm are potentially toxic. Although histamine occurs naturally in the human body, exposure to large doses can rapidly produce the symptoms of toxicity.

Outbreaks and Incidence

The symptoms of histamine poisoning resemble an allergic reaction, and there is potential for misdiagnosis. Furthermore, since symptoms are usually mild, it is likely that the illness is considerably under-reported. Nevertheless, it is thought that histamine poisoning is probably the commonest form of fish-related toxicity. The highest numbers of cases are reported in the USA, Japan and the UK, but this may be a reflection of reporting systems rather than incidence. Between 1992 and 2004, England and Wales reported 56 outbreaks affecting 296 people. Outbreaks were more common in summer than in winter. In the USA, between 1968 and 1980, 103 outbreaks involving 827 people were reported and in Japan over the same period, 42 outbreaks affecting 4,122 people. Large outbreaks also occur. In 1973, at least 200 US consumers became ill after eating domestic canned tuna. In the first 6 months of 2005, an unusual increase in incidence was reported in England and Wales, with 16 outbreaks affecting 38 people. This was thought to be associated with poor temperature control and hygiene in certain catering premises.

Sources

Histamine in fish and other foods is produced by the decarboxylation of the amino acid histidine, and fish species that have high levels of free histidine in their tissues are most likely to develop toxic histamine levels. This is usually the result of the action of the enzyme histidine decarboxylase, which is found in a number of bacterial species that may occur on fish. Species such as *Vibrio* spp., *Pseudomonas* spp. and *Photobacterium* spp. are found in the marine environment and occur naturally on fish. Others, especially the Enterobacteriaceae, are contaminants that are introduced after postharvest. It is this second group that is considered most important in the development of histamine. Species such as *Morganella morganii*, *Klebsiella pneumoniae* and *Hafnia alvei* are able to produce high levels of histamine very rapidly at mesophilic temperatures (20–30°C). For this reason, histamine is more often produced during spoilage in this temperature

range, although high levels can also develop at lower temperatures over time.

In tropical waters the indigenous microflora may be more important histamine-producing organisms, particularly when fishing methods such as longlining are used, where the fish may die before landing. Under these conditions, it is possible for histamine to be formed before the fish is landed and chilled. There is evidence that histidine decarboxylase remains active at chill temperatures, even though the bacteria themselves are not active. Therefore, once the enzyme has been formed at higher temperatures, it may continue to produce histamine even when the fish is properly chilled. It is also possible for histamine to form after cooking or canning if the fish subsequently becomes contaminated with histidine decarboxylase-producing bacteria. This can happen when canned fish is handled under conditions of poor hygiene.

Stability in Foods

Histamine is extremely stable once formed and is not affected by cooking. It can survive canning and retorting processes and is not reduced during freezing or frozen storage. Furthermore, high histamine levels may not be accompanied by other signs of spoilage and may be undetectable other than by chemical analysis. The enzyme histidine decarboxylase is inactivated by cooking, and further histamine will not then be produced unless recontamination occurs.

4.8.3 Control Options

Temperature Control

Chilling

The key measure for the control of histamine production in fish is rapid chilling as soon as possible after death, particularly where the fish has been exposed to warm water. This will inhibit the formation of bacterial histidine decarboxylase. Once the enzyme is present, control options are very limited. Accepted guidelines (FAO/FDA) recommend that fish should be placed in ice or chilled seawater or brine at $<4.4°C$ within 12 h of death or placed in chilled seawater or brine at $<10°C$ within 9 h of death. If the fish have been exposed to air or water temperatures above 28.3°C, they should be chilled to $<4.4°C$ within 6 h, and very large fish such as tuna that are eviscerated before chilling also should have the body cavity packed with ice.

Further chilling to a temperature as close to the freezing point as possible is desirable to prevent less rapid formation of histidine decarboxylase at lower temperatures. Even rapid chilling to $<4.4°C$ may only give a safe shelf life of 5–7 days. Once frozen, the fish can be stored safely for extended periods, and further histidine decarboxylase will not be formed. However, enzyme produced before freezing will not be destroyed and will continue to produce histamine after thawing.

Cooking

Cooking will destroy both histamine-producing bacteria and bacterial decarboxylases, but not histamine itself. Cooked fish therefore can be stored safely for longer periods, and canned fish can be kept almost indefinitely. It is important to note that once cooked or canned fish becomes recontaminated with histamine-producing bacteria, temperature control again becomes critical to prevent a hazard. For example, canned tuna that is not consumed immediately after opening should be stored at $<5°C$ as soon as possible.

Good Hygienic Practice

Good hygienic practice on board fishing vessels, especially during landing and processing, is important to minimise contamination with non-indigenous histamine-producing bacterial species. Careful handling of fish to avoid damage to muscle tissue is also important in preventing contamination. For example, puncture wounds in fish can introduce contaminating bacteria into deep tissue where large concentrations of histidine are available. Histamine production may then happen much more quickly. Good hygiene at processing and preparation stages further along the supply chain, such as cutting and packing or in catering operations, is also important to prevent contamination of fresh fish or recontamination of frozen and cooked fish.

Chemical Testing

Histamine is only detectable by chemical analysis, and affected fish may appear otherwise satisfactory. Chemical testing can provide some assurance that toxic levels of histamine are not present, but the variability in histamine levels in a single fish means that very large numbers of samples must be taken. For this reason, chemical testing cannot be relied upon to demonstrate adequate control of the hazard, but can be useful as an HACCP verification tool.

Legislation

European legislation states that fish species belonging to families known to contain large amounts of histidine (e.g. Scombridae, Clupeidae) in their tissues should be tested for the presence of histamine. Nine samples should be tested from each lot, and the mean value should be $\leq$100 ppm. The lot is considered unsatisfactory if more than two samples give results between 100 and 200 ppm or if any sample gives a result of $\geq$200 ppm. A mean level of 200 ppm and a maximum limit of 400 ppm are permitted for fish that have undergone enzyme maturation in brine. In USA the Food and Drug Administration has issued guidelines for tuna and related fish establishing a 'defect action level' of 50 ppm in any sample. This is said to be indicative of spoilage and may mean that toxic levels are present in other samples. A separate toxicity level of 500 ppm is also given. The international Codex standard for fish also includes histamine levels as indicators of decomposition and hygiene and handling. A maximum average level of not more than 100 ppm is considered satisfactory in relation to decomposition, while an upper limit of 200 ppm in any one sample is applied for hygiene and handling.

4.9 Kainic Acid

Kainic acid is a natural marine acid present in some seaweed. It is a specific agonist for the kainate receptor used as an ionotropic glutamate receptor which mimics the effect of glutamate. Along with quisqualate, it is used in experiments to distinguish a receptor from the other ionotropic receptors for glutamate such as NMDA and AMPA (Fig. 11.4).

4.9.1 Occurrence

In 1953, kainic acid was originally isolated from the seaweed called 'Kainin-sou' or 'Makuri' (*Digenea simplex*) in Japan. 'Kainin-sou' is used as an anthelmintic in Japan. Kainic acid is a potent central nervous system stimulant and has been developed as the prototype neuroexcitatory amino acid for the induction of seizures in experimental animals, at a typical dose of 10–30 mg/kg in mice. Kainic acid is neuroexcitotoxic and epileptogenic, acting through specific kainate receptors. Because of the supply shortage in 2000, the price of kainic acid has risen significantly.

1. Antiworming agent
2. Neuroscience research
3. Neurodegenerative agent
4. Modelling of epilepsy
5. Modelling of Alzheimer's disease

It is present in *Digenea simplex* (red alga). Chemically, it is a cyclic amino acid and causes depolarisation of crayfish and insect muscle. It is used as a pharmacological tool.

4.10 Ibotenic Acid

Ibotenic acid (Fig. 11.5) is a chemical compound that is naturally occurring in the mushrooms *Amanita muscaria* and *Amanita pantherina*, among others. Ibotenic acid is a powerful neurotoxin that is used as a 'brain-lesioning agent' (Becker et al. 1999; Isacson et al. 1984) and has shown to be highly neurotoxic when injected directly into the brains of mice and rats.

4.10.1 Psychopharmacology

Unlike muscimol (the main psychoactive constituent of *Amanita muscaria*), which produces sedative–hypnotic effects and dissociative hallucinations (similar to zolpidem and other z-drugs at high doses), ibotenic acid's psychoactivity is not completely established and does not contribute in any known way to the effects of *Amanita muscaria* other than serving as a prodrug to muscimol.

4.10.2 Use in Research

Ibotenic acid is used as a brain-lesioning agent in the research environment. When injected intracranially, ibotenic acid causes the development of excitotoxic lesions of the brain. This method of experimental brain lesioning may be preferable in certain circumstances because, while it destroys neuron bodies in a particular area, tracts that cross through the target nucleus are not damaged.

It is produced by the fungi *Amanita muscaria* and *A. pantherina*. Their consumption causes CNS depression, ataxia, hallucinations, myoclonic twitchings and seizures.

Agent	Incubation period (Hr)	Food sources	Diagnosis
Bacillus cereus	2–8	Boiled, fried rice, powdered milk	Gram stain/ culture of food, faeces, vomitus
Clostridium botulinum	2–150	Cooked meat, fowl, gravy	Toxin assay from food, stool or serum, Gram +ve sporulating Bacillus
C. perfringens	12	Cooked meat, fowl, gravy	Food, faeces examination
Campylobacter sp.	72	Water, raw milk, meat	Stool culture (not lethal)
E. coli (invasive)	10	Unknown	Res. Lab (not known)
Salmonella sp.	12–36	Meat, poultry, milk, salads	Meat, poultry, milk, salads
Staphylococcus	2–4	Custards, gravy, ham	Gram stain of faeces, vomitus, toxin assay (Enterotoxin: heat stable, not lethal)
Streptococcus A	36	Meat, raw silk	May cause appendicitis
Vibrio cholerae	48	Water, raw food	Stool culture, special medium
Non cholera Vibrio	12	Raw, shellfish, water	Stool, body fluid, tissue culture
Yersinia enterocolitica	36	Meat, raw silk,	May cause appendicitis
Hepatitis A virus	20–30 days	Shellfish, salads, cold food	LFT or serology
Parvovirus 'Norwalk virus'	16–18 days	Shellfish	Isolation or serology
Rotavirus 'winter vomiting disease'	48	Unknown	Isolation/ serology
Trichinella spiralis	4–30	Undercooked pork, bear meat	Muscle biopsy, serology
Giardia lamblia	1–6 weeks	Water, salads	Stool examination
E. histolytica	20–30 days	Raw fruits and vegetables	Stool examination/ serology
Scombrotoxin	<1	*Proteus morganii* (bacteria) action on dead fish	Analysis of fish, faeces, vomitus for histamine
Ciguatoxin	3–10	Bottom-feeding shore fish	None
Shellfish toxin	<1	Molluscs	Bioassay
Mushrooms fast (muscarinic), slow (cell destructive) hallucinogenic	0.5	Inocybe, clitocybe species	Analysis of mushrooms, urine or body fluid
	10–14	psilocybe, *Amanita muscaria*	
	½–1		

5 Toxins Produced by Microorganisms

Important toxin-producing organisms that enter the body via food or water and cause harm are as follows:

The community at large is simultaneously exposed to wide variety of pathogens, allergens and pollutants in air, water and food with qualitative and quantitative variations in exposure levels. In developing countries, the situation is complicated by a wide variety of predisposing conditions such as multiple factor exposure, protein and calorie malnutrition, genetically predetermined vulnerability, lack of adequate protection to high risk groups of women, children and elderly and geoclimatic factors. There are many factors which determine the susceptibility of the individual, some of which are described below:

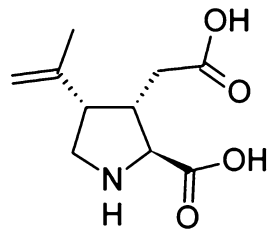

Fig. 11.4 Structure of kainic acid

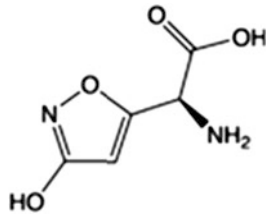

Fig. 11.5 Structure of ibotenic acid

Extrinsic factors affecting community health		
Source	Factors	Effects
Air	Aerosols, pathogens	Airborne bacterial and viral Diseases
Water	Pathogens	Waterborne diseases
	Biotoxins	Toxicity
Food	Biotoxins, biomagnified	Toxicity
	Pathogens	Diseases
	Allergens	Allergy

Intrinsic factors affecting community health	
Factors	Effects
Nutrition	
Protein/calorie deficiency	Diseased disease resistance, morbidity
Vitamin mineral deficiency	Anaemia etc.
Endemic factors	Metabolic changes
Genetic factors	Individual variations, atopy in airborne diseases, genotoxicity
Drug, tobacco and alcohol abuse	Toxicity

Biotoxins are produced by a wide range of organisms for defence or offence, or to catch prey, and they include animals and plants including microorganisms like bacteria and fungi. They can enter the body through ingestion of food and water or by air through aerosols. Marine biotoxins are important since they are most potent, and many of them get biomagnified and become part of food chain. Some toxins are used as drugs and/or pharmacological tools because of their specificity. The reaction of the body to a toxin depends upon many factors ranging from nutritional status to genetic make-up and dose of the toxin taken.

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Microbial Toxicity Studies

12

1 Introduction

Microbial toxins are toxins produced by microorganisms, including bacteria, viruses and fungi. Microbial toxins are important virulence determinants responsible for microbial pathogenicity and/or evasion of the host immune response. Some bacterial toxins, such as *botulinum* neurotoxins, are the most potent natural toxins known. However, microbial toxins also have important uses in medical science and research. Potential applications of toxin research include combating microbial virulence, the development of novel anticancer drugs and other medicines and the use of toxins as tools in neurobiology and cellular biology (Proft 2009). Botulinum neurotoxins (BoNTs) are the most potent natural toxins known. The family of BoNTs comprises seven antigenically distinct serotypes (A–G) that are produced by various toxigenic strains of spore-forming anaerobic *Clostridium botulinum*.

2 Bacterial Toxin

Bacterial toxin is a type of toxin that is generated by bacteria ('bacterial toxins' at Dorland's Medical Dictionary). They are classified as either exotoxin or endotoxin. Exotoxins are generated by the bacteria and actively secreted. Endotoxins are part of the bacteria itself. Usually, endotoxin is part of the bacterial outer membrane, and it is not released until the bacteria are killed by the immune system. The body's response to endotoxin can involve severe inflammation. In general, the inflammation process is usually considered beneficial to the infected host, but if the reaction is severe enough, it can lead to sepsis. Some bacterial toxins can be used in the treatment of tumours.

Toxinosis is caused by the bacterial toxin alone, not necessarily involving *Clostridium tetani*. *Clostridium tetani* produces tetanus toxin (TeNT protein), which leads to a fatal condition known as tetanus in many vertebrates (including humans) and invertebrates when the toxin is ingested. Toxinosis can be caused by *Staphylococcus aureus* toxins (Fisher et al. 2007).

2.1 *Botulinum* Neurotoxin

Botulinum neurotoxins (BoNTs) are the most potent natural toxins known. The family of BoNTs comprises seven antigenically distinct serotypes (A–G) that are produced by various toxigenic strains of spore-forming anaerobic *Clostridium botulinum*. They act as metalloproteinases that enter peripheral cholinergic nerve terminals and cleave proteins that are crucial components of the neuroexocytosis apparatus, causing a persistent but reversible inhibition of neurotransmitter release resulting in flaccid muscle paralysis. They are the causative agent of the deadly food poisoning disease, botulism, and could pose a major biological warfare threat due to their extreme toxicity and ease of

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production. They also serve as powerful tools to treat an ever-expanding list of medical conditions (Kukreja and Singh 2009).

2.2 Tetanus Toxin

Clostridium tetani produces tetanus toxin (TeNT protein) which leads to a fatal condition known as tetanus in many vertebrates (including humans) and invertebrates.

2.3 Anthrax Toxin

Bacillus anthracis produces two major virulence factors, a tripartite exotoxin referred to as anthrax toxin and an antiphagocytic capsule. These virulence factors mediate pathogen survival and, in the case of toxin, directly induce damage to the host. Two distinct enzymatic activities are associated with anthrax toxin, each encoded by a separate protein. The enzymatic subunits are lethal factor (LF), a zinc-dependent metalloproteinase, and oedema factor (EF), a calcium- and calmodulin-dependent adenylate cyclase. Lethal factor and EF gain access to the host cytosol by binding to and translocating through a pore formed by the shared binding subunit, protective antigen (PA). The combination of LF and PA is called lethal toxin (LT), and this toxin inactivates MAPK/ERK pathway in the host. Oedema toxin (ET), formed by the combination of EF and PA, produces high cAMP levels in host cells. Early during infection, systemic toxin levels are low and likely modulate the host immune response locally, thereby allowing establishment of infection. Late in infection, toxin concentration increases causing organ damage, vascular leakage and ultimately death of the host (Maldonado-Arocho et al. 2009).

2.4 Subtilase Cytotoxin

Subtilase cytotoxin (SubAB) is the recently recognised prototype of a new AB5 toxin family secreted by Shiga toxigenic *Escherichia coli* (STEC). Its A subunit is a subtilase-like serine protease, and cytotoxicity for eukaryotic cells is due to a highly specific, single-site cleavage of BiP/GRP78, an essential Hsp70 family chaperone located in the ER. This cleavage triggers a severe ER stress response, ultimately resulting in apoptosis. The B subunit has specificity for glycans terminating in the sialic acid N-glycolylneuraminic acid. The role of SubAB in human disease remains to be established (Paton and Paton 2009).

2.5 *Pasteurella multocida* Toxin

Pasteurella multocida toxin (PMT) is the major pathogenic determinant of *Pasteurella multocida*. The species *P. multocida* causes various diseases of animals and humans. The toxin is the causative agent of the economically important atrophic rhinitis in swine. Stimulation of several signalling pathways is induced by PMT. Most remarkable is a potent mitogenic effect. Phospholipase Cβ and the small GTPase Rho are activated due to stimulation of heterotrimeric G proteins of the Gαq and Gα12/13 family (Orth 2009).

2.6 *Vibrio* RTX Toxins

Multifunctional-autoprocessing RTX toxins are a unique family of secreted proteins toxins, predominantly produced by the *Vibrio* sp. The best characteristic of these toxins is produced by *V. cholerae*. In the eukaryotic cell, this toxin has three distinct biochemical activities resulting in autoprocessing, covalent cross-linking of actin and inactivation of Rho-family GTPases, ultimately resulting in destruction of the actin cytoskeleton. Related toxins produced by *V. vulnificus* and *V. anguillarum* have some similar mechanisms of action. These toxins may assist the bacterium to evade host immune defences (Satchell and Geissler 2009).

2.7 *Helicobacter pylori* Toxin

Helicobacter pylori, a gram-negative bacterium that colonises the human stomach, secretes a toxin known as VacA. This toxin was initially

identified on its ability to cause vacuolation in cultured gastric epithelial cells. VacA causes several other alterations in gastric epithelial cells and targets multiple types of immune cells. Most VacA-induced cellular alterations are attributable to insertion of the toxin into cellular membranes and the formation of membrane channels (Cover and Atherton 2009).

2.8 Staphylococcal Toxins

Immune evasion proteins from *Staphylococcus aureus* have a significant conservation of protein structures and a range of activities that are all directed at the two key elements of host immunity, complement and neutrophils. These secreted virulence factors assist the bacterium in surviving immune response mechanisms (Langley et al. 2009).

2.9 Cyanobacteria Toxins

Cyanobacteria produce a large variety of bioactive compounds, including substances with anticancer and antiviral activity, UV protectants, specific inhibitors of enzymes and potent hepatotoxins and neurotoxins (Herrero and Flores 2008).

2.10 Mycotoxins

In agriculture, *Aspergillus* originally was considered a serious problem largely because of its prevalence in the biodeterioration of stored crops and as an opportunistic pathogen of field crops, particularly under high-moisture conditions. During the early 1960s, the discovery of aflatoxins associated with massive deaths of poultry, trout and other domesticated animal species worldwide raised new awareness that these fungi posed threats to food and feeds beyond their ability to rot plant materials. Research on aflatoxins led to so-called golden age of mycotoxin research during which many new fungal toxins were discovered from species of *Aspergillus* and other common moulds. In addition to aflatoxins, other important *Aspergillus* mycotoxins include ochratoxin, patulin and fumigillin. Aflatoxins are still recognised as the most important mycotoxins. They are synthesised by only a few *Aspergillus* species of which *A. flavus* and *A. parasiticus* are the most problematic. The expression of aflatoxin-related diseases is influenced by factors such as age, nutrition, sex, species and the possibility of concurrent exposure to other toxins. The main target organ in mammals is the liver so aflatoxicosis is primarily a hepatic disease. Conditions increasing the likelihood of aflatoxicosis in humans include limited availability of food, environmental conditions that favour mould growth on foodstuffs and lack of regulatory systems for aflatoxin monitoring and control (Machida and Gomi 2010).

A. flavus and *A. parasiticus* are weedy moulds that grow on a large number of substrates, particularly under high-moisture conditions. Aflatoxins have been isolated from all major cereal crops and from sources as diverse as peanut butter and marijuana. The staple commodities regularly contaminated with aflatoxins include cassava, chillies, corn, cotton seed, millet, peanuts, rice, sorghum, sunflower seeds, tree nuts, wheat and a variety of species intended for human or animal food use. When processed, aflatoxins get into the general food supply where they have been found in both pet and human foods as well as in feedstocks for agricultural animals. Aflatoxin transformation products are sometimes found in eggs, milk products and meat when animals are fed with contaminated grains (Fratamico et al. 2008).

Human exposure to aflatoxins is difficult to avoid because *A. flavus* grows aggressively in many foods at all stages of the food chain: in the field, in storage and in the home. Evidence for acute human aflatoxicosis has been reported from several underdeveloped countries such as India and Thailand. The symptoms of severe aflatoxicosis include oedema, hemorrhagic necrosis of the liver and profound lethargy. Further, aflatoxins are potent carcinogens, especially aflatoxin B1. Based on epidemiological studies done in Asia and Africa, in 1988, the International Agency for Research on Cancer, part of the World Health Organization, placed aflatoxin B1 on the list of

human carcinogens. In developed countries, the emphasis on keeping aflatoxin out of the food chain concerns is carcinogenic potential. Strong regulatory limits (4–30 ppb) have been established for many commodities (Bennett 2010).

3 Fungal Ribotoxins

Ribotoxins are a family of fungal extracellular ribonucleases which inactivate ribosomes by specifically cleaving a single phosphodiester bond located at the universally conserved sarcin/ricin loop of the large rRNA. The subsequent inhibition of protein biosynthesis is followed by cell death via apoptosis. Ribotoxins are also able to interact with membranes containing acid phospholipids, their cytotoxicity being preferentially directed towards cells showing altered membrane permeability, for example, transformed or virus-infected cells.

4 Aquatic Microbiology

Bacteria in aquatic environments can be found in diverse habitats and communities. They are associated with all types of surfaces, including plants, rocks, animals, sediments, manufactured objects and plankton, and they are found in environments that have extreme physical and chemical ranges: temperatures −4 to +50 °C, salinity 0–1,000 atm, pH 5.5–8.5 and oxidation/reduction potential (Eh) +400 to −400 mV. The number, types and activities of these bacteria are basically dictated by their environmental setting. Concentration of organic and inorganic materials and temperature are probably the most restrictive factors controlling the growth of bacteria in aquatic habitats. Most bacteria are heterotrophs, deriving carbon and energy for growth from dead remains of plant and animal material. Dissolved organic food material from these remains is found in extremely low concentration (μg/l) in aquatic habitats. Thus, aquatic bacteria are efficient scavengers. Bacterial metabolism is geared to the utilisation of small concentrations of nutrients that appear intermittently. Bacteria associated with sediment and with plant and animal surfaces are often found in greater numbers per unit area than those that are free swimming. However, overall microbial activity may not reflect this mass difference because sediment and surface bacteria cells may be packed several cell layers thick. Those on-bottom layers are shielded from available nutrients and live essentially as dormant populations. Aquatic environments have a distribution of bacterial populations in which cell numbers decrease with distance from shore and with depth. Surface slicks on lakes and bays are frequently rich in bacterial biomass and metabolic activities compared to the water immediately below. The same is true of sediment water interface, where a loose slurry of sediment may contain many of the mineralisation activities associated with the aquatic environment. Thermoclines and euphotic zones of lakes and oceans can have areas with greater bacterial numbers and sometimes greater bacterial activities.

The principal activity of bacteria is the transformation of organically bound carbon, nitrogen, phosphorus, magnesium, sulphur and other elements into unbound oxidised states. This process is called mineralisation and is key to nutrient cycle in aquatic system. These nutrients provide plants with the essential factors needed for growth. Thus, the microbiologists and toxicologist are concerned with the toxic materials on bacteria and the processes they mediate. If changes in mineralisation and nutrient recycling are curtailed, the functioning of the ecosystem will be dramatically affected since the two are interlinked. Domestic sewage, for example, severely affects the microflora of waters. It changes the normal pattern of mineralisation and a number of economic consequences. The massive increase in nutrients associated with sewage pollution causes algal blooms. The algae eventually die, leaving a large pool of readily degradable organic matter which the bacteria try to eliminate it through mineralisation. In heavily affected areas bacteria degradative activities become limited, and with oxygen depletion large concentrations of sulphide accumulates thus killing fish and other animals. Specific toxicants can have similar effects. Thus,

it is important to assess routinely the potential impacts on aquatic bacteria and the processes they catalyse.

4.1 Role of Sediment Bacteria in Aquatic Ecosystems

Sediment microorganisms are crucial for the biodegradation of organic matter and the cycling of nutrients, while these microorganisms are susceptible to toxic pollutants. The degradation of organic pollutants in aquatic ecosystems is mainly performed by bacteria. Most of the bacteria in an aquatic ecosystem are bound to sediment particles. For example, 0.1 mm of a Dutch river sediment did contain as many bacteria as 10 m of water. Anaerobic conditions are common at the bottoms of lakes and slow-flowing rivers because the precipitation of organic material is high when the water current is low. The activity of the bacteria at the sediment surface rapidly degrades organic compounds and thereby generates an oxygen gradient. When oxygen is depleted sulphide can be formed which has a strong influence on the partitioning of metals and the degradation of organic compounds in the sediment. The depletion of oxygen in anaerobic sediments limits the occurrence of many animals and plants. The plants and animals which do occur in anaerobic sediments are specially adapted to obtain oxygen from the surface water or from the air. The effects of pollutants on the activity of bacteria at the sediment surface have not been studied in great detail, whereas it is vital for the health of the aquatic ecosystem. For the protection of the sediment ecosystem, one needs information on the sensitivity of the microorganisms, plants and animals which are living in and on the surface of sediments. Therefore, the results of toxicity tests with microorganisms, plants and animals may be combined in order to derive sediment quality guidelines. The use of the equilibrium partitioning method may be derived from sediment quality and water quality guidelines. When there are no results of toxicity tests with microorganisms, plants or animals available, it is very difficult to derive sediment quality guidelines. In the Netherlands these guidelines are derived from aquatic toxicity data using a sediment/water partitioning coefficient. The aquatic quality guideline (expressed in lg/l) is multiplied by the partitioning coefficient (expressed in l/kg) to obtain the sediment quality guideline (expressed in lg/kg). This procedure is unreliable when there is a large variation of partitioning coefficients for a single compound. Metals often show a large variation of sediment/water partitioning coefficients that depend on many factors like pH, clay content, organic matter content, iron content, sulphur content and redox potential. In the Netherlands there are a large number of aquatic sediments with a high clay content, a low redox potential and elevated metal concentrations. When dredging of these sediments is necessary, this can be a problem because they have to be treated as polluted sediments. The question remains open at which conditions the elevated metal concentrations in these sediments really pose an ecotoxicological risk.

4.2 Sediment Toxicity Tests to Derive Sediment Quality Guidelines

Sediment quality guidelines might also be derived from sediment toxicity tests with animals, plants or microorganisms that live in the sediment. The procedure would be similar to the derivation of soil quality guidelines. The concentration effect relation is summarised with a no observed effect concentration (NOEC) and an EC10 or EC50. The NOEC is the highest toxicant concentration that produces no significant difference with the control. The EC10 and EC50 are the toxicant concentrations that give 10 or 50 % inhibition. Many processes or enzymatic reactions can be monitored in sediment samples and can be used to obtain concentration effect relations. When the EC10 values of a specific toxicant for different processes and enzymatic reactions are collected, a microbial sensitivity distribution is obtained. The lowest EC10 value of this distribution may be taken to derive a safe concentration that can be used to set a sediment quality guideline. In practice, however, it is better to statistically derive the concentration that is

safe for 95 % of the processes and enzymatic reactions. This avoids excessively low values for the compounds for which many test results are available and relatively high values for the compounds for which only a few tests are available in the literature. Since the organisms are tested together with the sediment, it is not possible to attribute differences in sensitivity to the sediment properties or the properties of the microorganisms. It is always a combination of sediment properties and properties of microorganisms that determines sensitivity. Therefore, it has been suggested that each separate test should be used independently for the setting of sediment quality guidelines instead of grouping tests with the same process or function together as if it were tests with the same species. They also suggested that no sediment type correction should be performed since the microbial species and the sediment are tested together. This makes it impossible to separate species sensitivity and the bioavailability in the sediment. A similar procedure was described to derive sediment quality guidelines from toxicity tests with sediment microorganisms. It was shown that for 1,2-dichloroethane, chloroform and zinc, the quality guidelines derived from microbial toxicity tests in sediment were orders of magnitude lower than the quality guidelines derived from aquatic toxicity tests using the equilibrium partitioning method. For zinc this might be attributed to a high sensitivity of microbial toxicity tests or to uncertainties in the equilibrium partitioning constant. For 1,2-dichloroethane and chloroform this was caused by the high sensitivity of the anaerobic microbial processes.

Sediments contain clay particles, organic matter, iron oxides, sulphides and other compounds that can bind the toxicant and mitigate toxicity. The toxicity depends also on the pH and the presence of dissolved inorganic and organic compounds. This mitigation also occurs in soils. For the setting of the Dutch soil quality guidelines however, the soil pH is not taken into account although it has a pronounced influence on the toxicity of metals. The situation in sediments is even more complicated since also the amount of sulphide and the redox potential play an important role. These factors make it difficult to compare the toxicity of a compound in different sediments. Therefore, a scientifically underpinned sediment type correction will be difficult to obtain.

4.3 The Conditions in Unpolluted Anaerobic Sediments Can Be Toxic for Aerobic Organisms

While the presence of sediments can decrease toxic effects of pollutants, some of the naturally occurring compounds in anaerobic sediments can cause inhibitory effects. Animals and plants need oxygen for their metabolism that is obtained from the water or in the case of plants even from the air. The low oxygen concentrations and high sulphur and ammonia concentrations which naturally occur at the surface of anaerobic sediments can be inhibitory for plants or animals. The microorganisms that live in anaerobic sediments are well adapted to these concentrations. In some cases these microorganisms are so well adapted to anaerobic conditions that they are not able to survive in the presence of molecular oxygen. This illustrates the general principle that toxicity is not a substance property only but it is the combination of the substance, the organisms, the conditions and the exposure duration that can cause toxic effects.

4.4 The Use of Pollution-Induced Community Tolerance

Pollution-induced community tolerance (PICT) for the determination of sediment quality guidelines PICT can be caused by the following chain of events: The organisms in polluted sediments are exposed to elevated concentrations of pollutants. When the pollution exceeds a critical level, the most sensitive organisms become inhibited by toxic effects. This causes a decreased fitness in these organisms which can then be outcompeted by other more tolerant organisms. Therefore, the absence of sensitive species can be used as an indicator for the toxic

effects of a certain pollutant. The occurrence of PICT is often accompanied by a loss of species diversity. The tolerance of the organisms extracted from the sediment is measured under controlled laboratory conditions without sediment and is commonly expressed as the EC50 (in mg/l). For the comparison of the tolerance of the organisms, it is necessary to separate the organisms from the sediment in order to distinguish microbial tolerance from sorption to the sediment. The difference in tolerance between the microorganisms from a control site and a polluted site can give information about the percentage of the original microflora that has been affected at the polluted site.

4.5 A Separate Approach for Microbial Toxicity Tests Necessary for Ecotoxicological Risk Assessment of Polluted Sediments

Microorganisms do not form a separate taxonomic group like vertebrates or angiosperms since they are only defined as creatures which are too small to be seen by the naked eye. There are, however, taxonomic groups like the gram-positive bacteria or the cyanobacteria that contain only microbial species. Microorganisms do not grow more rapidly than plants or animals. The predominant microorganisms in soil, sediments and surface water do not grow rapidly. They have doubling times in the order of magnitude of weeks. Therefore, there is no need to treat microorganisms differently from animals or plants, when performing ecotoxicological risk assessment. Accordingly, the microbial tests with *Vibrio fischeri* or single species of algae were used together with the tests with fish or invertebrates to derive quality guidelines for aquatic ecosystems. There is however a large number of microbial toxicity tests which focus on the functions and processes that these microorganisms support. These tests are quite different from single-species tests and are therefore treated in a separate way. These functional tests are often combined to form a sensitivity distribution which is different from the sensitivity distribution of single-species tests. This functional sensitivity distribution is subsequently used in a risk assessment which leads to a separate ecotoxicological risk level for microbial functions. Subsequently, the lowest risk level of either the single-species tests or the functional tests is used to determine the ecotoxicological quality guidelines.

5 Effects of Toxicants on Microbial Growth

Population growth is relatively easy to measure in the laboratory. It can be studied in natural mixed populations, synthetic gnotobiotic populations or pure cultures. Some researchers consider that pure cultures are preferable for toxicity studies in certain situations; others think that mixed populations should be studied. Growth studies are generally simple and can employ a variety of experimental conditions related to specific physical and chemical conditions related to aquatic environments. Microbial populations spanning a range of physiological conditions and ecosystem types are easily accessible. Samples of sediments, soil, water, plants and other materials can be transported to the laboratory. With little additional effort, growth of microbial populations can be observed under laboratory conditions, and the effect of toxicant can be measured. Thus, initial estimates of toxicity involve growth studies.

6 Enumeration of Bacteria in Aquatic Environments

To perform toxicity studies on bacteria, it is necessary to be able to enumerate the bacteria in a specific population from a specific habitat and qualitatively measure metabolic activity. Due to their size, bacteria present unique problems that may make toxicity studies difficult to interpret. For example, live bacterial cells are commonly enumerated by spreading a dilute water sample on an agar medium that will support growth. If individual cells are separated

from their neighbours, they will grow and divide, eventually producing a visible colony. The number of colonies equals the number of bacteria present in the diluted water sample. This is a mechanically simple exercise, but the interpretation is confusing. First, there is no guarantee that cells may have originated from a single cell. It may have aggregated during or prior to plating. If the toxicant provoked aggregation, the result would seem to be reduction in cell numbers and an apparent toxicant-mediated mortality. Second, a toxicant may have bacteriostatic effect so that once it is washed away, the effect disappears. Enumeration techniques involving agar often ensure a constant concentration of toxicant, resulting in a misleading reduction in numbers not seen in nature. Thirdly, the agar medium should allow growth of all bacteria in the water sample. In most situations, however, only a small percentage of the total bacteria in the sample will grow on particular medium. With the diversity of metabolic types in natural bacterial populations, it is virtually impossible for all types to grow on a single type of medium. Thus, in a toxicity test, one never knows whether the bacteria that did not grow on a particular medium were more sensitive to the toxicant. An alternative to counting viable cells on agar is counting bacteria directly with microscope. This technique has been improved with the advent of epifluorescence counting (Daley and Hobie 1975). Cells are stained with acridine orange, a fluorescing dye that associates tightly with DNA and can be observed under a fluorescence microscope. The bacteria stand out vividly from the background, and all bacteria in sample can easily be counted. However, many bacterial cells can remain intact even though functionally dead. Consequently, it is difficult. Several variations on the epifluorescence technique have been used to differentiate live from dead bacteria. Nalidixic acid, for example, interferes with RNA synthesis in growing cells, causing them to enlarge and change shape; this allows live bacteria to be distinguished morphologically from dead cells (Orndroff and Colwell 1980). This technique may prove useful in toxicity tests with bacteria.

Direct counts invariably yield higher numbers of cells per unit volume than do plate counts, because dead cells and metabolically dormant cells are all counted together. However, direct counting has advantages, particularly in relation to microbial activity measures. Several other methods for enumerating bacteria are also available, but their application to toxicity testing is limited. Bacterial growth in broth media causes increased turbidity which can be quantitated with a spectrophotometer. The turbidometric method has been extensively used in microbiology, particularly with pure cultures. It is a very simple, reproducible technique. The classical multiphase growth response of bacterial cultures, logarithmic growth phase, stationary phase and depth phase can be routinely assessed. This technique is also valuable when studying the phases of growth that are most sensitive to a toxicant. However, growth phases are essentially a laboratory artefact; caution is required in extrapolating the results of the field.

Several biochemical components of bacterial cells can be quantitated and related to cell numbers. Measurements of cellular ATP levels, cell wall components such as lipopolysaccharide or muramic acid, lipid phosphate and other lipid components, nuclear material such as DNA and RNA and certain enzyme levels have been used with moderate success. In most cases, except for muramic acid and lipopolysaccharide, the measurements are not unique to bacteria. The presence of one algal cell or protozoan can greatly affect the concentration of these components and produce very misleading estimates of bacterial population densities. Muramic acid, which is found in the cell walls of cyanobacteria, often gives misleading information, since many cyanobacterial cells are considerably larger than most bacteria.

7 Growth of Heterotrophic Microorganisms

In the last decade, a couple of successful industrial heterotrophic microalgal productions have been established. Many microalgae can assimilate

organic substances to cover variable part of their carbon and energy requirements. To cover their entire energy requirements and be able to grow in complete darkness, the organic substances are respired in mitochondria with oxygen as electron acceptor, a process similar to the respiration in animal cells. Some algae, such as *Chlamydomonas*, may also use a slightly modified process to respire acetate, the so-called glyoxylate pathway. In *Chlamydomonas* the process is regulated so that it takes place only in the dark.

Chlamydomonas is also able to ferment starch, which was produced during the day, into ethanol under anaerobic conditions. So far, fermentation has been demonstrated only in a few microalgae species. Algae capable of growing in the dark are called true heterotrophs, while algae that require light but are able to supplement the metabolism with organic substances are called mixotrophs. Very few species, however, can grow in darkness in addition being able to grow also as true autotrophs—that is, in light without organic supplements.

Organic substances that may be respired include glucose, acetate, glycerol, TCA cycle intermediates and a number of amino acids. Only glucose, acetate and glycerol may play a role as substrates in industrial productions. These substances are small molecules, and algae are not normally able to metabolise large molecules such as proteins or even complex particles—but some algae, most notably in the classes Dinophyceae and Prymnesiophyceae, are able to engulf large molecules and particles in a process called pinocytosis or phagocytosis, depending on the size of particles.

Some dinophyceans have lost their ability to form chloroplasts but are able to retain functional chloroplast from ingested algae. A good place to look for heterotrophic species is among decaying seaweed where the decomposition processes result in a rich variety of dissolved organic substances.

The most common way to study the effects of toxic materials on microorganisms is to monitor growth inhibition on agar medium by a viable plate count. Dilutions of natural water samples are plated on agar medium that contains different concentrations of the toxicants, and the reduction in colony-forming units (CFUs) relative to control plates containing no toxicant are then observed. The plate count technique is based on the principle that each viable organism will give rise to one colony. This method is simple, economically suitable for statistical analysis and amenable to the examination of large number of water samples.

An example of this type of study is work on the toxicity of Kepone to estuarine microorganism (Mahaffey et al. 1982; Bourquin et al. 1978). Kepone is an organochlorine pesticide which contaminated the James River estuary in Virginia. To determine its toxicity to microorganisms, samples of estuarine water from several sampling sites around Pensacola, Florida, were serially diluted and plated on Zobell's agar containing Kepone concentrations of 0.1–2.0 mg/l (ppm). A significant reduction in CFU relative to control plates was detected on plates containing 0.2 mg/l. Twenty-three different colonies were selected from these plates and developed as pure microbial cultures. These isolates were found to be sensitive to Kepone to varying degrees, but gram-negative isolates were generally least sensitive to these pesticides. Since Kepone in the James River is primarily found in the sediments, it was important to determine whether the anaerobic organisms found primarily in estuarine sediments were also affected by Kepone. Kepone was not nearly as toxic to anaerobically grown microorganisms. This difference is probably due to interference of Kepone with oxidation and respiration, similar to the mechanism described by Widdus et al. (1971) and Trudgill et al. (1971) for the toxicity of chlordane to Bacillus sp. Orndroff and Colwell (1980) compared the viable plate counting technique with direct counting methods. The acridine orange epifluorescence method developed by Daley and Hobie (1975) was used for direct counts of bacterial populations. In this method, acridine orange stains biological material gives a distinct green colour under fluorescence microscope. To obtain an indication of the number of live bacteria comprising total direct count, Orndoff treated the cells with nalidixic acid by the method of Kogure (1979). Nalidixic acid specifically inhibits RNA

synthesis in live gram-negative cells and causes a change in their shape, enabling them to be distinguished from dead cells. Since most aquatic microorganisms are gram-negative and Kepone is not toxic to gram-negatives, use of the Kogure technique is justified.

In all these studies it is important to perform a statistical analysis on the data to determine whether effects resulting from a toxicant exposure are significant. Several statistical analyses can be used for this purpose. For example, in the work by Mahaffey et al. (1982), the effect of Kepone on plate counts of bacteria was statistically analysed by a three-factor model analysis of variance (Zar 1974).

8 Geochemical Cycling: Nitrogen

In geography and Earth science, a biogeochemical cycle or substance turnover or cycling of substances is a pathway by which a chemical element or molecule moves through both biotic (biosphere) and abiotic (lithosphere, atmosphere and hydrosphere) compartments of Earth. A cycle is a series of change which comes back to the starting point and which can be repeated. The term 'biogeochemical' tells us that biological, geological and chemical factors are all involved. On the other hand the circulation of chemical nutrients like carbon, oxygen, nitrogen, phosphorus, calcium and water through the biological and physical world are known as biogeochemical cycle. In effect, the element is recycled, although in some cycles there may be places (called reservoirs) where the element is accumulated or held for a long period of time (such as an ocean or lake for water). Water, for example, is always recycled through the water cycle. The water undergoes evaporation, condensation and precipitation, falling back to Earth clean and fresh. Elements, chemical compounds and other forms of matter are passed from one organism to another and from one part of the biosphere to another through the biogeochemical cycles. Ecological systems (ecosystems) have many biogeochemical cycles operating as a part of the system, for example, the water cycle, the carbon cycle and the nitrogen cycle. All chemical elements occurring in organisms are part of biogeochemical cycles. In addition to being a part of living organisms, these chemical elements also cycle through abiotic factors of ecosystems such as water (hydrosphere), land (lithosphere) and/or the air (atmosphere).

The living factors of the planet can be referred to collectively as the biosphere. All the nutrients—such as carbon, nitrogen, oxygen, phosphorus and sulphur—used in ecosystems by living organisms are a part of a closed system; therefore, these chemicals are recycled instead of being lost and replenished constantly such as in an open system. The flow of energy in an ecosystem is an open system; the sun constantly gives the planet energy in the form of light, while it is eventually used and lost in the form of heat throughout the trophic levels of a food web. Carbon is used to make carbohydrates, fats and proteins, the major sources of food energy. These compounds are oxidised to release carbon dioxide, which can be captured by plants to make organic compounds. The chemical reaction is powered by the light energy of the sun. It is possible for an ecosystem to obtain energy without sunlight. Carbon must be combined with hydrogen and oxygen in order to be utilised as an energy source, and this process depends on sunlight. Ecosystems in the deep sea, where no sunlight can penetrate, use sulphur. Hydrogen sulphide near hydrothermal vents can be utilised by organisms such as the giant tube worm. In the sulphur cycle, sulphur can be forever recycled as a source of energy. Energy can be released through the oxidation and reduction of sulphur compounds (e.g., oxidising elemental sulphur to sulphite and then to sulphate).

Although the Earth constantly receives energy from the sun, its chemical composition is essentially fixed, as additional matter is only occasionally added by meteorites. Because this chemical composition is not replenished like energy, all processes that depend on these chemicals must be recycled. These cycles include both the living biosphere and the nonliving lithosphere, atmosphere and hydrosphere. The chemicals are sometimes held for long periods of time in one place.

This place is called a reservoir, which, for example, includes such things as coal deposits that are storing carbon for a long period of time. When chemicals are held for only short periods of time, they are being held in exchange pools. Examples of exchange pools include plants and animals. Plants and animals utilise carbon to produce carbohydrates, fats and proteins, which can then be used to build their internal structures or to obtain energy. Plants and animals temporarily use carbon in their systems and then release it back into the air or surrounding medium. Generally, reservoirs are abiotic factors whereas exchange pools are biotic factors. Carbon is held for a relatively short time in plants and animals in comparison to coal deposits. The amount of time that a chemical is held in one place is called its residence.

There are many biogeochemical cycles that are currently being studied for the first time as climate change and human impacts are drastically changing the speed, intensity and balance of these relatively unknown cycles. These newly studied biogeochemical cycles include:

- The mercury cycle
- The human-caused cycle of atrazine, which may affect certain species

Biogeochemical cycles always involve hot equilibrium states: a balance in the cycling of the element between compartments. However, overall balance may involve compartments distributed on a global scale.

As biogeochemical cycles describe the movements of substances on the entire globe, the study of these is inherently multidisciplinary. The carbon cycle may be related to research in ecology and atmospheric sciences. Biochemical dynamics would also be related to the fields of geology and pedology (soil study).

9 Decomposition Processes

Bacteria and fungi are primarily responsible for the decomposition of bulk organic matter produced in aquatic environments. Most of the organic matter comes from plant debris. It is either transformed into soluble organic substances and eventually mineralised to carbon dioxide or transformed into particulate organic carbon, which may become resistant to microbial breakdown. Accumulation of humic acids, pectins, lignins and chitin may be the result of the latter degradation processes. Microbial transformation requires a wide range of enzymes, including cellulases for plant material, lipases for lipids, amylases for starch, proteases for proteins, amidases for chitin and oxygenases for lignin. A single bacterial species probably will not have any genetic information for more than one or two of these enzymes. But these activities can be found in a typical interacting population of aquatic bacteria, where commensalistic and mutualistic coordination among bacteria and higher plants and animals can be found. A chemical that disrupts one of these enzymatic processes may ultimately alter the metabolic capability of the entire population.

There are two basic approaches to study toxic effects in anaerobic systems. One is to study a specific microbiological process, such as methane production. The other is to study the integrated activities of a large number of organisms and chemical processes.

10 Unique Aspects of the Effects of Toxicants on Microorganisms

A variety of toxicology studies differ considerably from norm, but the creativity and uniqueness merit special attention. These studies are not routine, but they offer new insights that may prove useful in future development of toxicity testing. They reflect some fascinating aspects of microbiology and may stimulate new thinking in the ecotoxicology of microbial process.

10.1 Chemotaxis

Bacteria are capable of tactic responses to particular chemicals, including toxicants. Young and Mitchell (1973) showed that toxicants can negatively affect chemotaxis in bacteria. Ordinarily

because bacteria are so small, this type of response would be difficult to observe. Hazelbauer 2012, however, developed a method in which a capillary pipette is filled with a chemical or nutrient source and placed in dilute culture of bacteria. After a short period of incubation, the mouth of the pipette is covered with bacterial cells. This attraction to a chemical is called positive chemotaxis. When a toxic chemical is included in a nutrient broth in the capillary, the bacteria do not accumulate near the tip or inside. This is termed as negative chemotaxis. Chemicals that neither stimulate nor inhibit chemotaxis are distinct because bacteria around the capillary tip are randomly distributed and no bacteria are found around the pipette.

10.2 Epiphytic Microorganisms

Almost any surface in the body of water has a layer of algae covering it. Invariably associated with the algae and adhering to them is population of epiphytic bacteria. In this symbiotic relationship, the algae excrete organic materials for bacterial growth, and bacterial metabolism supplies CO_2 and inorganic nutrients for algal growth. This symbiosis is important for the ecosystem and is desirable to know the extent to which a toxicant disturbs the community.

10.3 Bioaccumulation in Bacteria

Ko and Lockwood (1968) showed that chlorinated hydrocarbon pesticides were readily accumulated by pure cultures of soil fungi and actinomycetes. Grimes and Morrison (1975) extended the observation to aquatic bacterial isolates. Pure cultures were grown in nutrient broth, washed by centrifugation and resuspended in various pesticides at a concentration of 0.1 μg/l. At specific time intervals, subsamples were removed and centrifuged; the concentration of the pesticide in water was determined by gas chromatography. These factors were bioconcentrated by factors ranging from 10 for lindane to as much as 59,000 for chlordane.

10.4 Adverse Effects of Microorganisms on Toxic Substances

Toxicity of a chemical in an aquatic environment is coupled to its chemical and biological fate. Some very toxic chemicals are innocuous to the environment because their degradation to non-toxic by-products is rapid. Biodegradation is one of the principal fates of these chemicals, and humans depend on the metabolic diversity of microbial populations to ensure that many synthetic organic materials do not accumulate in the environment. Chemicals have been synthesised with structures that the enzymatic machinery of microorganisms cannot attack at significant rates leading to major pollution problems with chemicals like DDT, Kepone and polychlorinated biphenyls (PCBs)

It is emphasised that biodegradation may generate a product more toxic than the parent compound. Although this is not well documented, there are enough examples to concern microbiologists and to warrant studies of the toxicity of the degradation products. Mercury is probably the most publicised example of a substance that was more toxic after microbial transformation. The anaerobic activities of microorganisms in sediments caused methylation of mercury. As a result, the mercury was made more water soluble and was mobilised from the sediment and more thoroughly mixed with the water column. More toxic forms of the element showed that bacteria, through bioaccumulation of mercury and consumption by higher organism, can enhance the concentration of mercury in aquatic food chains. Methylation of elements (cadmium, arsenic, tin) by microorganisms can also potentially produce a more toxic by-product.

Crude oil contains many polynuclear aromatic hydrocarbons that are slowly degraded and could yield intermediate degradation products that are carcinogenic and mutagenic. Organophosphate insecticides have $P = S$ bonds and could be oxidised by bacteria to corresponding $P = O$ (oxone) derivatives, which have considerably greater mammalian toxicity. Bacteria participate in N-alkylation and nitrosamination leading to the formation of nitrosamines from a variety of

common precursors such as secondary amines and nitrites. Nitrosamines are powerful carcinogens and mutagens. Although many of these potential degradation products are not toxic to bacteria, bacterial activities lead to their formation.

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Toxic Cyanobacteria in Water and Their Public Health Consequences 13

1 Introduction

Cyanobacteria (blue-green algae) produce toxins that may present a hazard for drinking water safety. These toxins (microcystins, nodularins, saxitoxins, anatoxin-a, anatoxin-a(s), cylindrospermopsin) are structurally diverse, and their effects range from liver damage, including liver cancer, to neurotoxicity. The occurrence of cyanobacteria and their toxins in water bodies used for the production of drinking water poses a technical challenge for water utility managers. With respect to their removal in water treatment procedures, of the more than 60 microcystin congeners, microcystin-LR (L, L-leucine; R, L-arginine) is the best studied cyanobacterial toxin, whereas information for the other toxins is largely lacking. In response to the growing concern about nonlethal acute and chronic effects of microcystins, the World Health Organization has recently set a new provisional guideline value for microcystin-LR of 1.0 mg/L drinking water. This will lead to further efforts by water suppliers to develop effective treatment procedures to remove these toxins. These treatments may not be sufficient during bloom situations or when a high organic load is present, and toxin levels should therefore be monitored during the water treatment process. In order to perform an adequate human risk assessment of microcystin exposure via drinking water, the issue of water treatment by-products will have to be addressed in the future.

Cyanobacteria or blue-green algae occur worldwide especially in calm, nutrient-rich waters. Some species of cyanobacteria produce toxins that affect animals and humans. People may be exposed to cyanobacterial toxins by drinking or bathing in contaminated water. The most frequent and serious health effects are caused by drinking water containing the toxins (cyanobacteria) or by ingestion during recreational water contact.

2 The Disease and How It Affects People: The Cause

Disease due to cyanobacterial toxins varies according to the type of toxin and the type of water or water-related exposure (drinking, skin contact, etc.). Humans are affected with a range of symptoms including skin irritation, stomach cramps, vomiting, nausea, diarrhoea, fever, sore throat, headache, muscle and joint pain, blisters of the mouth and liver damage. Swimmers in water containing cyanobacterial toxins may suffer allergic reactions, such as asthma, eye irritation, rashes and blisters around the mouth and nose. Animals, birds and fish can also be poisoned by high levels of toxin-producing cyanobacteria.

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2.1 The Cause

Cyanobacteria are also known as blue-green algae, so named because these organisms have characteristics of both algae and bacteria, although they are now classified as bacteria. The blue-green colour comes from their ability to photosynthesise, like plants. Cyanobacterial toxins are classified by how they affect the human body. Hepatotoxins (which affect the liver) are produced by some strains of the cyanobacteria, namely, *Microcystis*, *Anabaena*, *Oscillatoria*, *Nodularia*, *Nostoc*, *Cylindrospermopsis* and *Umezakia*. Neurotoxins (which affect the nervous system) are produced by some strains of Aphanizomenon and Oscillatoria. Cyanobacteria from the species *Cylindrospermopsis raciborskii* may also produce toxic alkaloids, causing gastrointestinal symptoms or kidney disease in humans. Not all cyanobacteria of these species form toxins, and it is likely that there are as yet unrecognised toxins. People are mainly exposed to cyanobacterial toxins by drinking or bathing in contaminated water. Other sources include algal food tablets. Some species form a scum on the water, but high concentrations may also be present throughout the affected water. Surface scums, where they occur, represent a specific hazard to human health because of their particularly high toxin contact. Contact, especially by children, should be avoided.

3 Distribution and Scope of Problem

The organisms can grow rapidly in favourable conditions, such as calm nutrient-rich fresh or marine waters in warm climates or during the late summer months in cooler parts of the world. Blooms of cyanobacteria tend to occur repeatedly in the same water, posing a risk of repeated exposure to some human populations. Cyanobacterial toxins in lakes, ponds and dugouts in various parts of the world have long been known to cause poisoning in animals and humans. One of the earliest reports of their toxic effects was in China 1,000 years ago (Sivonen and Jones 1999).

Cyanobacteria have been linked to illness in various regions throughout the world, including North and South America, Africa, Australia, Europe, Scandinavia and China. There are no reliable figures for the number of people affected worldwide. The only documented and scientifically substantiated human deaths due to cyanobacterial toxins have been due to exposure during dialysis. People exposed through drinking water and recreational water have required intensive hospital care.

4 Steps to Prevent Cyanobacterial Poisoning

1. Reducing nutrient buildup (eutrophication) in lakes and reservoirs, especially by better man agreement of wastewater disposal systems and control of pollution by fertilisers (including manure) from agriculture.
2. Educating the staff in the health and water supply sectors, as well as the public, about the risks of drinking, bathing or water sports in water likely to contain high densities of cyanobacteria.
3. Water treatment to remove the organisms and their toxins from drinking water supplies, where appropriate.
4. Algae are vitally important to marine and freshwater ecosystems, and most species of algae are not harmful. Algal blooms occur in natural waters used for drinking and/or recreation when certain types of microscopic algae grow quickly in water, often in response to changes in levels of chemicals such as nitrogen and phosphorus from fertiliser, in the water. Algal blooms can deplete the oxygen and block the sunlight that other organisms need to live, and some can produce toxins that are harmful to the health of the environment, plants, animals and people. Harmful algal blooms (HAB) have threatened beaches, drinking water sources and even the boating venue for the 2008 Olympic Games in

Beijing, China. Cyanobacteria (blue-green algae) and red tides are examples of algae that can bloom and produce toxins that may be harmful to human and animal health. HABs can occur in marine, estuarine and freshwaters, and HABs appear to be increasing along the coastlines and in the surface waters of the United States, according to the National Oceanic and Atmospheric Administration (NOAA). HSB epidemiologists have led a number of studies to investigate the public health impacts of blue-green algae blooms and Florida red tide. The studies have demonstrated that there is potential for exposure to potent HAB-related toxins during recreational and occupational activities on water bodies with ongoing blooms.

5. Although scientists do not yet understand fully how HABs affect human health, authorities in the United States and abroad are monitoring HABs and developing guidelines for HAB-related public health action. The US Environmental Protection Agency (EPA) has added certain algae associated with HABs to its Drinking Water Contaminant Candidate List. This list identifies organisms and toxins that EPA believes are priorities for investigation.
6. Many states regularly experience harmful algal blooms (HABs), and state public health departments are often asked to provide guidance about HAB-associated human and animal illnesses. HSB subject matter experts help states to develop their public health responses to HAB events, including providing outreach and education materials and assessing exposure and the potential for health effects.

5 Cyanobacteria (Blue-Green Algae)

Cyanobacteria, also known as blue-green algae, grow in any type of water and are photosynthetic (use sunlight to create food and support life). Cyanobacteria live in terrestrial, fresh, brackish or marine water. They usually are too small to be seen but sometimes can form visible colonies, called an algal bloom. Cyanobacteria have been found among the oldest fossils on earth and are one of the largest groups of bacteria. Cyanobacteria have been linked to human and animal illnesses around the world, including North and South America, Africa, Australia, Europe, Scandinavia and China.

6 Cyanobacterial Blooms and How They Are Formed

Cyanobacterial blooms (a kind of algal bloom) occur when organisms that are normally present grow exuberantly. Within a few days, a bloom of cyanobacteria can cause clear water to become cloudy. The blooms usually float to the surface and can be many inches thick, especially near the shoreline. Cyanobacterial blooms can form in warm, slow-moving waters that are rich in nutrients such as fertiliser runoff or septic tank overflows. Blooms can occur at any time but most often occur in late summer or early fall. They can occur in marine, estuarine and fresh waters, but the blooms of greatest concern are the ones that occur in freshwater, such as drinking water reservoirs or recreational waters. Some cyanobacterial blooms can look like foam, scum or mats on the surface of freshwater lakes and ponds. The blooms can be blue, bright green, brown or red and may look like paint floating on the water. Some blooms may not affect the appearance of the water. As algae in a cyanobacterial bloom die, the water may smell bad.

7 Harmful Marine Algae

Harmful marine algae, such as those associated with red tides, occur in the ocean and can produce toxins that may harm or kill fish and marine animals. There are many kinds of marine algae that produce toxins that can accumulate in shellfish. In USA, one of the illnesses that may result from eating algal toxin-contaminated shellfish is neurotoxic shellfish poisoning (NSP). NSP is

caused by eating shellfish contaminated with brevetoxins, which are produced by *Karenia brevis*, the marine algae associated with Florida red tides. NSP is a short-term illness with neurologic symptoms (such as tingling fingers or toes) and gastrointestinal symptoms. There are very few cases of NSP in the USA because coastal states carefully monitor their shellfish beds and close the beds to harvesting if high concentrations of brevetoxins are detected in the water or the shellfish. Brevetoxins may also be in the air along the Gulf coast of Florida during Florida red tide events, and many symptoms such as eye irritation and a sore throat occur in healthy people. People who have asthma may have symptoms, such as chest tightness, that last for several days after exposure. Ciguatera tides fish poisoning is another disease associated with toxins produced by marine algae. The toxin responsible, called ciguatoxin, accumulates through the food web, and very high levels may exist in reef fish, particularly (but not only) large carnivorous reef fish.

8 Red Tide

Algae are vitally important to marine ecosystems, and most species of algae are not harmful. However, under certain environmental conditions, microscopic marine algae called *Karenia brevis* (*K. brevis*) grow quickly, creating blooms that can make the ocean appear red or brown. People often call these blooms 'red tide'.

Karenia brevis produces powerful toxins called brevetoxins, which have killed millions of fish and other marine organisms. Red tides have damaged the fishing industry, shoreline quality and local economies in states such as Texas and Florida. Because *K. brevis* blooms move based on winds and tides, pinpointing a red tide at any given moment is difficult. Red tides occur throughout the world, affecting marine ecosystems in Scandinavia, Japan, the Caribbean and the South Pacific. Scientists first documented a red tide along Florida's Gulf Coast in fall 1947, when residents of Venice, Florida, reported thousands of dead fish and a 'stinging gas' in the air, according to Mote Marine Laboratory. However, Florida residents have reported similar events since the mid-1800s.

9 Assessing the Impact on Public Health

In addition to killing fish, brevetoxins can become concentrated in the tissues of shellfish that feed on *K. brevis*. People who eat these shellfish may suffer from neurotoxic shellfish poisoning, a food poisoning that can cause severe gastrointestinal and neurologic symptoms, such as tingling fingers or toes. The human health effects associated with eating brevetoxin-tainted shellfish are well documented. However, scientists know little about how other types of environmental exposures to brevetoxin—such as breathing the air near red tides or swimming in red tides—may affect humans. Anecdotal evidence suggests that people who swim among brevetoxins or inhale brevetoxins dispersed in the air may experience irritation of the eyes, nose and throat, as well as coughing, wheezing and shortness of breath. Additional evidence suggests that people with existing respiratory illness, such as asthma, may experience these symptoms more severely.

10 Ciguatera

Ciguatera fish poisoning (or ciguatera) is an illness caused by eating fish that contain toxins produced by a marine microalgae called *Gambierdiscus toxicus*. Barracuda, black grouper, blackfin snapper, cubera snapper, dog snapper, greater amberjack, hogfish, horse-eye jack, king mackerel and yellowfin grouper have been known to carry ciguatoxins. People who have ciguatera may experience nausea, vomiting and neurologic symptoms such as tingling fingers or toes. They also may find that cold things feel hot and hot things feel cold. Ciguatera has no cure. Symptoms usually go away in days or weeks but can last for years. People who have ciguatera can be treated for their symptoms.

11 Toxic Compounds Produced by Cyanobacteria

Cyanobacterial toxins are generally divided into categories based on their principal modes of action in mammalian systems. Further, research on cyanobacterial compounds shows that these are low molecular weight compounds which show adverse biological activities in a range of toxicity-based systems involving aquatic animals, cells and enzymes.

1. *Neurotoxin*
   The most widely known group of carbamate toxins is known as saxitoxins. These are more commonly known as products of marine red tides of dinoflagellate algae. They are known for Paralytic Shellfish Poisoning (PSP), and a group of 21 structurally related saxitoxin variants is currently recognised (Fig. 13.1). The major mode of action regarding toxicity in vertebrates is via blockage of voltage-gated sodium channels, resulting in paralysis and in acute cases death.
2. A second group of cyanobacterial neurotoxins is named after *anatoxin-a*. This toxin is a secondary amine and its molecular mode of toxic activity is a postsynaptic acetylcholine antagonist, resulting in paralysis, asphyxiation and death. It is a naturally occurring organophosphate molecule. Anatoxin-a(S) is a unique phosphate ester of a cyclic N-hydroxyguanine (MW = 252) produced by *Anabaena flos-aquae* strain NRC 525-17. It has more recently been identified in blooms and isolated strains of *Anabaena lemmermannii*. The LD50 of anatoxin-a(S) is 20 μg kg-1 bw (i.p. mouse) (Carmichael 1997). Structural variants of anatoxin-a(S) have not been detected (Fig. 13.2).
3. *Hepatotoxins*
   Cylindrospermopsin
   Cylindrospermopsin is a toxin produced by cyanobacteria, or blue-green algae, that has severe effects on the liver and other organs. The structure is shown in Fig. 13.3.

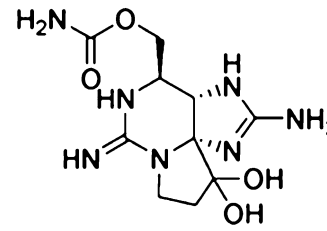
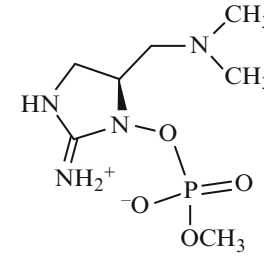


Fig. 13.1 Saxitoxin

Fig. 13.2 Anatoxin-a



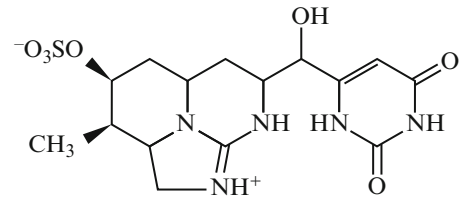


Fig. 13.3 Cylindrospermopsin

11.1 Microcystin

Globally the most frequently found cyanobacterial toxins in blooms from fresh and brackish waters are the cyclic peptide toxins of the microcystin and nodularin family. They pose a major challenge for the production of safe drinking water from surface waters containing cyanobacteria with these toxins. In mouse bioassays, which traditionally have been used to screen toxicity of field and laboratory samples, cyanobacterial hepatotoxins (liver toxins) cause death by liver haemorrhage within a few hours of the acute doses. Microcystins have been characterised from planktonic *Anabaena*, *Microcystis*, *Oscillatoria* (Planktothrix), *Nostoc* and

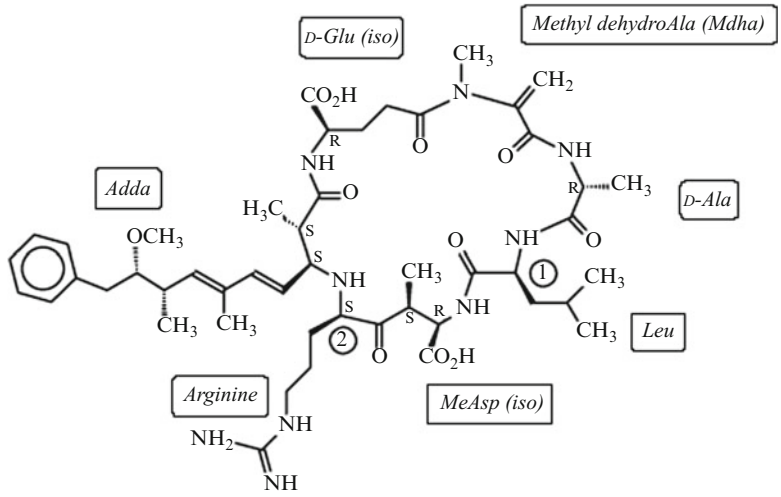


Fig. 13.4 The generic structure of a microcystin. Variations occur primarily at positions 1 and 2. For example, Microcystin-LR contains the amino acids leucine (*L*) and arginine (*R*) at positions 1 and 2, respectively. Microcystin-RR has arginine at both positions. Nodularins are similar, with the five amino acids Adda-γGlu-Mdhb-β MeAsp-Arg making up the core ring system

Anabaenopsis species, and from terrestrial *Hapalosiphon* genera. Nodularin has been characterised only from *Nodularia spumigena*.

The cyclic peptides are comparatively large natural products, molecular weight (MW) approximately 800–1,100, although small compared with many other cell oligopeptides and polypeptides (proteins) (MW > 10,000). They contain either five (nodularins) or seven (microcystins) amino acids, with the two terminal amino acids of the linear peptide being condensed (joined) to form a cyclic compound. They are water soluble, excepting a few somewhat more hydrophobic microcystins, are unable to penetrate directly in the lipid membranes of animal, plant and bacterial cells. Therefore, to elicit their toxic effect, uptake into cells occurs through membrane transporters which otherwise carry essential biochemicals or nutrients. This restricts the target organ range in mammals largely to the liver. In aquatic environments, these toxins usually remain contained within the cyanobacterial cells and are only released in substantial amounts on cell lysis. Along with their high chemical stability and their water solubility, this containment has important implications for their environmental persistence and exposure to humans in surface water bodies.

The first chemical structure of cyanobacterial cyclic peptide toxins was identified in the early 1980s, and the number fully characterised toxin variants has greatly increased during the 1990s. The first such compounds found in freshwater cyanobacteria were cyclic heptapeptides (i.e. they contain seven peptide-linked amino acids) with the general structure of cyclo-(D-alanine1-*X*2-D-MeAsp3-Z4-Adda5-D-glutamate6-Mdha7) in which X and Z are variable L amino acids, D-MeAsp3 is D-erythro-ß-methylaspartic acid, and Mdha is N-methyldehydroalanine. The amino acid Adda, (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, is the most unusual structure in this group of cyanobacterial cyclic peptide toxins (Fig. 13.4). These compounds were first isolated from the cyanobacterium *Microcystis aeruginosa*, and therefore the toxins was named microcystins. Structural variations have been reported in all seven amino acids, but most frequently with substitution of L-amino acids at positions 2 and 4, and demethylation of amino acids at positions 3 and/or 7. About 60 structural variants of microcystins have been characterised so far from bloom samples and isolated strains of cyanobacteria.

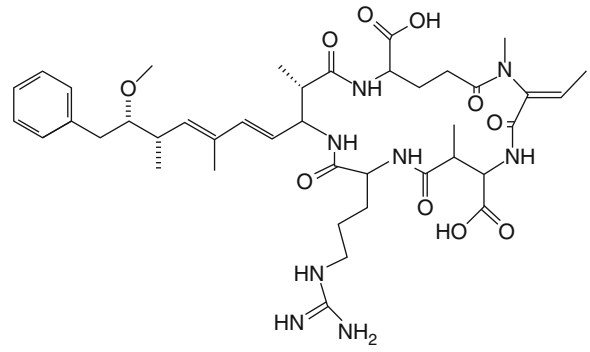


Fig. 13.5 Nodularin

11.2 Nodularin

In one species of brackish water cyanobacterium, an identically acting and structurally very similar cyclic pentapeptide occurs. It has been named as nodularin after its producer, *Nodularia spumigena*. The chemical structure of nodularin is cyclo-(D-MeAsp1- L-arginine2-Adda3-D-glutamate4-Mdhb5), in which Mdhb is 2-(methylamino)-2- dehydrobutyric acid. A few naturally occurring variations of nodularins have been found: two demethylated variants, one with D-Asp1 instead of D-MeAsp1, the other with DMAdda3 instead of Adda3; and the nontoxic nodularin which has the 6Zstereoisomer of Adda3.

The equivalent 6Z-Adda3 stereoisomer of microcystins is also nontoxic (Fig. 13.5). In the marine sponge *Theonella swinhoei*, a nodularin analogue called motuporin has been found. It differs from nodularin only by one amino acid, having hydrophobic L-Val in place of the polar L-Arg in nodularin. The toxin might be cyanobacterial in origin because the sponge is known to harbour cyanobacterial symbionts.

11.3 Lipopolysaccharides

Lipopolysaccharides (LPS), also known as lipoglycans, are large molecules consisting of a lipid and a polysaccharide joined by a covalent bond (Fig. 13.6). They are found in the outer membrane of gram-negative bacteria, act as endotoxins and elicit strong immune responses in animals. LPS is the major component of the outer membrane of gram-negative bacteria, contributing greatly to the structural integrity of the bacteria and protecting the membrane from certain kinds of chemical attack. LPS also increases the negative charge of the cell membrane and helps to stabilise the overall membrane structure. It is of crucial importance to gram-negative bacteria, whose death results if it is mutated or removed. LPS is an endotoxin and induces a strong response from normal animal immune systems. It has also been implicated in nonpathogenic aspects of bacterial ecology, including surface adhesion, bacteriophage sensitivity and interactions with predators such as amoebae.

LPS is required for the proper conformation of its activity. However, smooth LPS will sterically hinder omptins. LPS acts as the prototypical endotoxin because it binds the CD14/TLR4/MD2 receptor complex, which promotes the secretion of proinflammatory cytokines in many cell types but especially in macrophages and B cells. In immunology, the term 'LPS challenge' refers to the process of exposing a subject to an LPS that may act as a toxin. LPS is also an exogenous pyrogen (external fever-inducing substance). Being of crucial importance to gram-negative bacteria, these molecules make candidate targets for new antimicrobial agents.

Some researchers doubt reports of generalised toxic effects attributed to all lipopolysaccharides, in particular, for cyanobacteria (Stewart et al. 2006).

It comprises of three parts:

1. O antigen (or O polysaccharide)
2. Core oligosaccharide
3. Lipid A

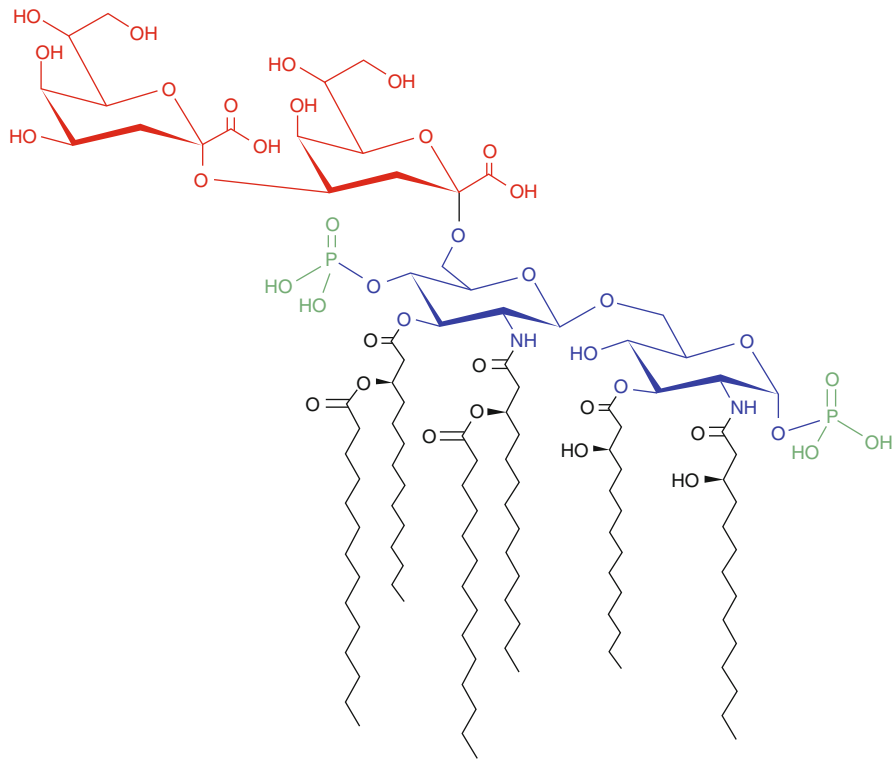


Fig. 13.6 Lipopolysaccharides

11.3.1 O Antigen

A repetitive glycan polymer contained within an LPS is referred to as the O antigen, O polysaccharide or O side chain of the bacteria. The O antigen is attached to the core oligosaccharide and comprises the outermost domain of the LPS molecule. The composition of the O chain varies from strain to strain. For example, there are over 160 different O antigen structures produced by different *E. coli* strains (Raetz and Whitfield 2002). The presence or absence of O chains determines whether the LPS is considered rough or smooth. Full-length O chains would render the LPS smooth, whereas the absence or reduction of O chains would make the LPS rough (Rittig et al. 2004). Bacteria with rough LPS usually have more penetrable cell membranes to hydrophobic antibiotics, since a rough LPS is more hydrophobic. O antigen is exposed on the very outer surface of the bacterial cell and, as a consequence, is a target for recognition by host antibodies.

11.3.2 Core

The core domain always contains an oligosaccharide component that attaches directly to lipid A and commonly contains sugars such as heptose and 3-deoxy-D-mannooctulosonic Acid (also known as KDO, keto-deoxyoctulosonate) (Hershberger and Binkley 1968). The LPS Cores of many bacteria also contain non-carbohydrate components, such as phosphate, amino acids and ethanolamine substituents.

11.3.3 Lipid A

Lipid A is, in normal circumstances, a phosphorylated glucosamine disaccharide decorated with multiple fatty acids. These hydrophobic fatty acid chains anchor the LPS into the bacterial membrane, and the rest of the LPS projects from the cell surface. The lipid A domain is responsible for much of the toxicity of gram-negative bacteria. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever,

diarrhoea and possible fatal endotoxic shock (also called septic shock).

11.4 Other Bioactive Compounds

Cyanobacteria produce a wide range of novel products with biological activities which include from enzyme inhibition to skin and gastrointestinal irritation. These are low molecular weight compounds and include microvirdins, anabaenopeptolins, microginins, cyanobacterins, fischerellins and nostocyclamides. Mostly they are not accurately toxic to higher animals but include products which have toxic effects on developmental and digestive functions of zooplanktons and with potential to act as grazing deterrents. Several cyanobacteria which grow as shoreline mats in tropical and subtropical waters produce irritant toxicants including aplysiatoxin, debromoaplysiatoxin and lyngbyatoxin-A which present health hazards to swimmers and include tumour producers.

12 Method of Detecting and Quantifying Cyanobacterial Toxins

The detection and quantification of cyanobacterial toxin is necessary to:

(a) Provide understanding of their occurrence and abundance in natural waters and potable supplies
(b) Investigate their toxicities and roles of waterborne health incidents
(c) Contribute to risk management of waterbodies affected by cyanobacterial abundance

12.1 Physicochemical Methods

A wide range of methods are used to detect and quantify saxitoxins, anatoxin-a and its analogues, microcystins, nodularins and cylindrospermopsins (Meriluto 1997; Codd et al. 2001). High-performance liquid chromatography (HPLC) has been the most widely used tool.

Liquid chromatography (LC) detection system includes the use of fluorescence. This has been used to detect further variants of anatoxin-a and saxitoxin which do not permit detection by parent compound. Other physicochemical methods for the analysis of certain microcystin and nodularin variants include electrochemical techniques employing cyclic voltammetry. However, this procedure is suitable for those variants that contain arginine and tyrosine residues such as microcystins-LR_1-RR and YR.

MS offers improved sensitivity for the analysis of anatoxin and derivatives as compared to UV-based absorbance systems. M systems such as fast atom bombardment mass spectrometry (FAB-MS) are increasingly been used to elucidate toxin structure, and some methods offer the possibility to analyse multiple toxin classes present in the same sample. Presently, LC-MS systems and matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) offer the possibility to detect cyanotoxin variants, although capital outlay for such system is currently high. One potential advantage of MALDI-TOF, although not a quantitative method, is that it can detect a wide array of cyanobacterial products. This has resulted in the production of peptide toxin mass peak libraries which are proving useful cyanobacterial toxin.

12.2 Biological, Biochemical and Immunological Methods

Traditionally, cyanobacterial toxins have been detected and quantified according to toxicity which they exhibit in mammalian test systems. The mouse bioassay holds an important role in the detection of cyanobacterial hepato- and neurotoxins. The mouse bioassay is still an Association of Official Analytical Chemists (AOAC) approved method for the analysis of marine

saxitoxins in contaminated shellfish and is legislated for the same purpose in EC laws. Currently, *in vitro* system exists for detection and quantification of saxitoxins, anatoxin-a, cylindrospermopsins and microcystin and nodularins.

Other *in vitro* systems use antibodies generated against the parent toxin in immunoassay (Codd et al. 2001; Metcalf and Codd 2003). Antibodies against microcystins and saxitoxins have been developed, and results show good correlation with traditional methods of analysis such as HPLC. As the cyanobacterial toxins have molecular weights of less than about 1,000 Da, these compounds are known as haptens, in that they are unable to invoke an immune response to produce specific antibodies against these compounds and also drastically reduce their toxicity before immunisation.

Cyanobacterial LPS can be quantified by haemagglutination assays. The most common method is the Limulus amoebocyte lysate (LAL) assay (Anderson et al. 2002; Rapala et al. 2002). Now a number of newly developed systems, such as use of chromogenic substrates, are allowing more accurate LPS quantification. One of the challenges to risk management of LPS problems associated with cyanobacterial blooms is to determine the relative contribution to the cyanobacterial LPS, versus that of co-occurring bacteria, to overall LPS toxicity.

12.3 Concentration of Cyanobacterial Toxins for Analysis

When cyanobacterial blooms, scums and mats are present, cyanobacterial toxin concentrations are applicable to direct analysis methods such as HPLC-PDA. However, when cyanobacteria-free or cyanobacterial toxin-containing water samples require analysis, concentration procedures may be required to permit toxin detection by such methods. Solid Phase Extraction (SPE) procedures have been successfully applied to meet these needs. For microcystins, nodularins and anatoxin-a, the use of C_{18}SPE material has allowed successful retention of these cyanotoxins from water matrices. Recent immunoaffinity methods have shown that 15 purified microcystins and nodularins can be successfully retained from water samples with >80% recovery (Arando et al. 2003). This use of immunological reagents against the cyanobacterial toxins could have wide and straightforward application for the recovery of the toxins from water for analysis.

12.4 Determination of the Potential for Cyanobacterial Toxin Production

Increasing research is elucidating the mechanisms of cyanobacterial toxin synthesis. Although the microcystins are peptides, these are synthesised by enzymic mechanisms, rather than via the classical biological mechanism of peptide and protein synthesis involving ribosomal RNA (Kaebernick and Neilan 2001). By analogy with other peptides and alkaloids produced enzymically by microorganisms, genetic methods such as PCR are increasingly being applied to detect the presence of genes for production of microcystins, saxitoxin and cylindrospermopsin by cyanobacteria. However, although genes are responsible for the production of cyanobacterial toxins are known and many methods for analysis are available, only the potential for toxin production can be thereby assessed, and depending on the information required, for example, end users and regulatory bodies, toxin analysis by more traditional methods may be necessary.

13 Cyanobacterial Toxins as Hazard to Health: Human and Animal Poisoning Episodes

It is well established that cyanobacterial toxins present hazards to human and health. Mortalities of sheep, cows, horses, pigs, dogs, poultry and fish have occurred as a result of ingestion of cyanobacterial scum, met or bloom material. Microcystins, anatoxin-a, saxitoxins, nodularin, cylindrospermopsin and anatoxin (a) have been identified as causative agents, either alone or in combinations. The most recent and serious known human poisoning episode occurred in

Brazil in 1996. Water from a drinking water reservoir, which had recently experienced cyanobacterial blooms, was tinkered to a haemodialysis clinic where it was effectively treated and then administered to haemodialysis patients. As a result, 126 patients were severely affected and 60 patients eventually died over a number of months. About 86% experienced toxic symptoms including tender hepatomegaly and biochemical evidence of liver injury. Severely affected patients also showed a range of neurological impairments. In UK soldiers who indulged in barrel rolling and swimming and canoeing training exercises in water containing Microcystis scum experienced gastrointestinal illness and mucosal membrane blistering with severe atypical pneumonia and indicators of liver damage requiring hospitalisation. The dermal route of cyanobacterial intoxication is responsible for severe contact dermatitis conditions such as 'swimmers itch' and can occur when people swim in sea in contact with cyanobacteria. Other risk activities include showering in ineffectively treated water and during work practices where dermal and respiratory exposure to cyanobacterial blooms and toxins may occur.

14 Animal Dosing Studies and Risk Assessment for Production of Human Health

Although there is large amount of information on the toxicity of individual, purified cyanobacterial toxins, little is known about the effects of multiple dosing. For example, intranasal exposure of mice to microcystin-LR was found to have a toxicity ten times greater than that of oral administration of the toxin by gavage. Examination of the nasal cavities revealed extensive necrosis of the olfactory and respiratory zone epithelium. In the same study, anatoxin-a was also administered with microcystin-LR intranasally, and synergistic effects were noted (Fitzgeorge et al. 1994).

15 Effect of Cyanobacterial Toxins on Wild Animals and Plants

Cyanobacterial mass populations can have adverse effects on wildlife in addition to humans and domestic livestock (Codd 1995; Carmichel 1997; Falconer 1998; Sivonen and Jones 1999). A wide range of wild animals (mammals, amphibians, fish, invertebrates and birds) have been affected with consequences from nonfatal to fatal. Wild animal poisoning can occur after incidental ingestion of cyanobacterial biomass and toxins during drinking or feeding. In the case of fish, it is possible that additional exposure can occur via the gill surfaces. Cyanobacterial blooms containing μg to mg per litre concentrations of cyanobacterial toxins pose health risks to adults and juveniles of aquatic vertebrates and invertebrates. Aquatic plants can take up microcystins at environmentally encountered concentrations. Phytoplanktonic birds have known to feed on cyanobacteria as a major or sole food source. Their pin plumage is a result of the ingestion of cyanobacterial pigments.

16 Multiple Fate of Cyanobacterial Toxins

Cyanobacterial toxins undergo multiple fates after biosynthesis (Sivonen and Jones 1999). These are not only of biological interest but of potential for the management of cyanobacterial toxin problems. When cyanobacterial cells are actively growing and healthy, the microcystin pools are mostly retained within the producer cells. However, with cylindrospermopsin, for example, even during growth, a large portion of the total pool may occur in external water. However, extracellular release of cyanobacterial toxins is accelerated during cell lysis, such as during natural bloom decay and in water treatment processes if these disrupt the cyanobacterial cells by physical or chemical

action. Even when cyanobacterial toxins have been released, they can potentially persist for long periods. These small molecules are nonvolatile and relatively stable. Microcystins can withstand boiling and extremes of pH. However, in natural environments, they are subject to photodegradation by UV and visible light and biodegradation by a range of naturally occurring harmless bacteria.

17 Dose of Cyanobacterial Toxins

Awareness of the properties and production of cyanobacteria and their toxins is a necessary part of risk management of cyanobacterial problem in the health, recreation, amenity, agriculture, aquaculture and drinking water supply (NRA 1990).

The risk management of cyanobacterial toxins includes reactive and proreactive measures (NRA 1990; NSWBGATF 1992; Yoo et al. 1995). Regarding reactive actions, the effectiveness of traditional and advanced drinking water treatment processes for the removal/destruction of cyanobacterial toxins is receiving attention with encouraging results for microcystins, anatoxin-a and cylindrospermopsin. The removal of cyanobacterial LPS (Rapala et al. 2002) has been demonstrated with 59–97% reduction caused by conventional treatments such as coagulation, setting and sand filtration. Reactive measures include the formulations of decision-making systems, including emergency measures for access to and use of waterbodies in the event of cyanobacterial populations and toxins having developed to unacceptable concentrations (NRA 1990; NSWBGATF 1992; Yoo et al. 1995; Scottish Executive 2002).

Proactive measures needed are basic knowledge of cyanobacterial toxin toxicity, production and persistence. This is necessary to permit the further confident derivative of drinking water GVs for additional toxins. Since policy development for cyanobacterial toxin risk management follows closely behind the primary research and developing awareness of the health significance and impacts of the toxins, it is necessary for effectiveness and suitability of action plans to be reviewed periodically and if necessary modified. This rolling approach is necessary modified.

Cyanobacterial toxins and their undesirable effects are being increasingly seen as part of the consequences of eutrophication (FWR 2000). Measures to reduce later, for example, restricting the excessive enrichment of water resources due to agricultural runoff and inadequately treated sewage now being addressed from local to catchment level, are seen as important longer-term actions which will contribute to cyanobacterial risk management.

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Good Laboratory Practices in Biomonitoring

14

1 Introduction

In the experimental research arena, the phrase good laboratory practice or GLP specifically refers to a quality system of management controls for research laboratories and organisations to try to ensure the uniformity, consistency, reliability, reproducibility, quality and integrity of chemical (including pharmaceuticals) non-clinical safety tests, from physiochemical properties through acute to chronic toxicity tests.

GLP was instituted after animal tests were not positive by pharmaceutical and industrial chemical (mainly pesticide) manufacturers. Industrial Bio-Test Labs (IBT) was the most notable case, where thousands of safety tests for chemical manufacturers were falsely claimed to have been performed or were so poor that police investigators could not piece together what work had been done even though IBT superficially delivered the test results their contracts with the manufacturers specified (Schneider 1983). The original GLP regulatory mandate was promulgated in 1978 by US-FDA and published in the Federal Register 43 FR 59985-60020. It was followed by a few years later by US-EPA (as outlined in the Organisation for Economic Co-operation and Development (OECD) Principles of GLP in 1992). The OECD has since help promulgate it to many countries, helping them to place it into their national regulations. GLP applies to non-clinical studies conducted for the assessment of the safety or efficacy of chemicals (including pharmaceuticals) to man, animals and the environment. Good laboratory practice (GLP) embodies a set of principles that provide a framework within which laboratory studies are planned, performed, monitored, recorded, reported and archived. These studies are undertaken to generate data by which the hazards and risks to users, consumers and third parties, including the environment, can be assessed for pharmaceuticals (only preclinical studies), agrochemicals, cosmetics, food additives, feed additives and contaminants, novel foods, biocides, detergents, etc. GLP helps to assure regulatory authorities that the data submitted are a true reflection of the results obtained during the study and can therefore be relied upon when making risk/safety assessments. GLP, a data quality system, should not be confused with standards for laboratory safety—appropriate gloves, glasses and clothing to handle laboratory materials safely.

2 GLP and OECD

GLP is a quality system concerned with the organisational processing process and conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported (OECD principles 1998).

GLP principles include:

(a) Organisation and personnel
(b) Management responsibilities
(c) Sponsor responsibilities

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(d) Study director responsibilities
(e) Principal investigator responsibilities
(f) Study personnel responsibilities
(g) Quality assurance programme
(h) Quality assurance personnel
(i) Facilities
(j) Test system facilities
(k) Facilities for test and reference items
(l) Equipments, reagents and materials
(m) Test systems
(n) Physical/chemical
(o) Biological
(p) Test and reference items
(q) Standard operating procedures
(r) Performance of study
    - Study plan
    - Conduct of study
(s) Reporting of results
(t) Storage of records and reports

3 OECD Guidelines for the Testing of Chemicals

OECD publishes OECD Guidelines for the Testing of Chemicals, which are guidelines that usually have to be followed for GLP compliance. They are widely required by agencies doing risk assessments of chemicals.

4 GLP and the USFDA

Preclinical trials on animals in the United States of America use these rules prior to clinical research in humans. Research in the USA not conducted under these restrictions or research done outside USA not conducted according to the OECD Guidelines (or FDA rules) might be inadmissible in support of a New Drug Application in the USA.

5 GLP and the European Union

Since 1987 the European Council had adopted two basic Directives and a Decision relating to the application of the GLP principles. "Directive 2004/10/EC" of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances.' This "Directive 2004/10/EC" lays down the obligation of the member states to designate the authorities responsible for GLP inspections in their territory. It also comprises requirements for reporting and for the internal market.

'Directive 2004/9/EC of the European Parliament and of the Council of 11 February 2004 on the inspection and verification of good laboratory practice (GLP)':

The Directive requires that the OECD Revised Guides for Compliance Monitoring Procedures for GLP and the OECD Guidance for the Conduct of Test Facility Inspections and Study Audits must be followed during laboratory inspections and study audits:

- 89/569/EEC Council Decision of 28 July 1989 on the acceptance by the European Economic Community of an OECD decision/recommendation on compliance with principles of good laboratory practice

There are also 'Product-Oriented Directives' referring to GLP obligations:

- REACH Regulation of 18 December 2006 and Directive 2006/121/EC of 18 December 2006
- Medicinal products; Directive 2001/83/EC on the Community code relating to medicinal products for human use of 6 November 2001 as amended by Commission Directive 2003/63/EC
- Veterinary medicinal products; Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products
- Cosmetics; Council Directive 93/35/EEC amending for the 6th time directive 76/768/EEC
- Feeding stuffs; Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition

- Foodstuffs; Directive 89/107/EEC
- Novel foods and novel food ingredients; Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients
- Pesticides; Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market
- Biocides; Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market
- Detergents; Directive 98/8/EC Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents
- EC eco-label; Commission Decision 2005/344/EC of 23 March 2005; establishing ecological criteria for the award of the Community eco-label to all-purpose cleaners and cleaners for sanitary facilities

In the meantime the EU has concluded Mutual Acceptance Agreements in the area of GLP with Israel, Japan and Switzerland. By means of the Treaty of the European Economic Area of 13 September 1993, the European Regulations and Directives also apply to Iceland, Liechtenstein and Norway.

6 GLP and Non-OECD Member Countries

An inspection in non-member economies by OECD inspectors will not guarantee that data generated in compliance with GLP will be accepted in other member countries than the one to which they are submitting data and which has thus sent inspectors to verify the accuracy of their compliance statement.

7 Criticism of GLP

GLP studies require adequate and permanent documentation of everything involved in an experimental test (staff qualifications, valid study design, standard operating procedures (SOPs), training, performance, formulation and statistical analysis and the retention of summary/individual data) so that there can be confidence in the study design, performance and its results and anyone (as public agencies have access to the GLP records) can subsequently fully reconstruct the study. GLP is by most regulatory authorities worldwide adopted as the lowest common standard for quality assurance. ISO 17025, GMP or GCP criteria are alternatives in some cases.

OECD Guideline test methods are recommended by regulatories as study plan to follow for toxicology studies. These methods are all very standardised/extensively peer reviewed and are adopted worldwide. Independent of the test guidelines, GLP is recommended by the authorities to assure the correct execution of these study plans. The correct execution of GLP study is verified by an independent GLP monitoring authority on a regular basis. This verification means an *in situ* inspection of the whole test facility and connected test sites worldwide. Audits of the studies registered with unrestricted access to all raw data produced during the whole study are a part of the inspection. In this sense it means a much deeper peer review of the study is done for an academic publication.

By contrast, academic scientists perform a wider range of basic/exploratory experimental research to identify unknown potential hazards of chemicals, elucidate the mode/mechanism of action for known toxicants and explore novel toxic end points. Accordingly, their experimental methods vary greatly in the delivery route of the test chemical, the number of test animals and the range of doses (Tweedale 2011). These test methods are far more varied than the GLP test protocol is and academics do not like to share their results or methods with laboratories competing for granting money or to give insight in raw data produced. These factors make it hard for regulatory agencies to use the results of academic researchers in chemical risk assessment.

The problem is the regulatory agencies universal requirement that toxicity studies be performed according to OECD/GLP protocols automatically excludes the toxicity results of

the independent researchers. The latter's methods, though variable, do test more realistic doses than the OECD protocols use. Thus, if they find toxicity at lower doses, that important risk is not included in the risk assessment, due to the GLP requirement. Tens of thousands of published findings of toxicity from chronic toxicity have been excluded from risk assessment, a large fraction of which find toxicity at lower dose than OECD tests.

Reviews of toxicity studies have confirmed that this false-negative error is common: Dozens of reviews have confirmed it for Guideline tests of pharmaceuticals, while for chemicals at least four reviewers have found it. In one of these, the toxicity studies funded by the manufacturers of a high-volume and well-studied chemical never found low-dose toxicity, but over 90% of its many government-funded studies did (vom Saal and Hughes 2005). The specific factors that lead to such false-negative error by OECD/GLP studies have been analysed (Myers et al. 2009).

8 Klimisch Score

The Klimisch score system tries to rank the reliability of toxicity studies for use by risk assessors (regulatory agencies). It was published in 1997, by BASF (a chemical company) authors (Klimisch et al. 1997). Studies performed according to GLP are assigned the top rank of 1 (reliable without restriction) and are preferred by agencies. When no GLP study is available for a particular end point, a study with a rank of 2 is usually accepted by an agency. Lower ranks typically require a new study to be performed. Klimisch scoring is very widely used in chemical risk assessments. Critics say it is a self-interested bias on objectivity and that a quality system from the regulated party gives their own GLP-complying studies the top rank.

9 GLP and Automated Systems

In many instances, the optimal recommended 'no-argument' means of implementing GLP is to develop an automated approach to both sample preparation and sample measurement. If this can include an overarching 'chain of custody' sample history and data flow, combined with adequate SOP's for calibration and linearisation of measuring tools, GLP compliance is virtually assured.

10 Good Clinical Laboratory Practices (GCLP)

Good clinical laboratory practices (GCLP) should be used by all laboratories where tests are done on biological specimens for diagnosis, patient care, disease control and research. All over the world the laboratories use GCLP to improve the quality of their work, to improve patient care given by clinicians and also to improve safety of staff who work in the laboratories. Implementation of GCLP is a stepwise process of meticulous planning, perfect execution with involvement by the whole team of laboratory personnel. Although many laboratories in India do follow some measures of good laboratory practices.

The laboratories in our country can be brought under three categories: primary care, secondary and tertiary level laboratories. In addition there are also reference laboratories and research laboratories. Therefore, each laboratory should align themselves with the category they belong, depending upon the scope of work the laboratories should have the facilities according to their needs.

11 Infrastructure

Infrastructure of laboratories should be planned according to the services provided by the laboratory. The basic infrastructure facilities include:

(a) Reception room/area where requisition forms are received and reports dispersed.
(b) Specimen collection room/area, toilets, privacy for special purposes, for example, semen collection, facilities for disabled persons, toilet for staff.

(c) Quality water supply for analytical purpose.
(d) Uninterrupted power supply.
(e) Analytical work area.
(f) Specimen/sample/slide storage facility including cold storage where applicable.
(g) Record room/area.
(h) Facility for cleaning of glassware, sterilisation/disinfection.
(i) Waste disposal facility including biomedical wastes.
(j) Fire safety equipment.
(k) Ventilation, climate control and lighting arrangements.
(l) Separate room for meetings/administrative work.
(m) Separate facilities/area for staff for hand washing, eating and storing food, drinks, etc.
(n) Communication facility with referral centres.
(o) Transport of specimen/samples to referral centres.
(p) Additional infrastructure facilities may be added for special tasks as and when needed.

12 Personnel, Training and Development

Every laboratory should have properly qualified staff at various levels depending upon the nature of the work. The qualification and experience of staff are well documented in NABL document 2007. Every staff member should be given a job description and should be trained to do the job that they are assigned to perform. The laboratory/management should also provide continuous professional development and training for the staff. There are well-accepted promotional avenues and polices and these should be made available to all the staff of the laboratory.

13 Equipment

The laboratories should be appropriately equipped for the task that they are going to perform. Unnecessary fancy equipments should be avoided and only appropriate technology and instruments should be installed. Care should be taken to install the instruments in suitable locations to facilitate smooth flow of samples. Regular maintenance and cleaning should be performed. The operating manual should be available for all the staff members. Maintenance contract, contact telephone number of service engineers and good logbook should be provided for the troubleshooting and maintenance of the equipment. Equipment should be properly calibrated, and the performance should be verified by running internal and external quality control samples. All calibrations of equipments including pipettes and thermometers should be performed only by authorised personnel and documented. Accreditation agencies, such as NABL, require that calibration certificates issued at prescribed intervals.

14 Reagents, Chemicals and Consumables

All reagents used in the laboratory should be of certain certified standards. The reagents, chemicals and consumables should be stored under appropriate environmental conditions. When new reagent lots are introduced, they should be validated by using control/reference materials and this should be documented. All reagents should be properly labelled including concentration, date of preparation/reconstitution, expiry date and storage conditions. The water used by the laboratory should be of prescribed quality.

15 Specimen Collection

The patient should be properly counselled before the specimen collection and consent should be taken whenever needed. A lot of pre-analytical errors can happen if care is not taken during the specimen collection. The phlebotomist, nurses and doctors who collect samples should be trained periodically in sample collection. The lab should prepare a ‘primary specimen collection manual’

containing information on patient preparation, methodology for specimen collection, labelling and transporting the samples. Necessary instructions on the preservative to be used, storage and transportation conditions should be provided.

16 Requisition Form

The requisition form for testing should be written only by the doctor and sent along with the sample. This should contain patient identity, age, sex, date and time of sample collection and investigation requested. A brief clinical history of the patient would be helpful to the laboratory. The lab should ensure that the unique ID number (hospital number) for the patient is stuck/written on the tube.

17 Registration of Samples

When samples for analysis are received by the laboratory, the laboratory should note down the time of specimen receipt and all details like name, age, sex, location in the hospital or medical unit the patient is attached to, name of physicians, investigation requested and date. There should be a column for remarks where the condition of specimen can be recorded. The laboratory should assign a unique registration number and then the process of analysis can start.

18 Worksheet

Nowadays, many analyzers can read barcode labels and the ID number, tests requested, etc. could be printed and stuck on the tube. Every laboratory should also provide a worksheet to the analyst wherein the test requested with a column for signature of the person analysing the sample and other relevant data can be entered. The request form and the worksheet can also be combined and made into a single form.

19 Reporting of Test Results

Test report data should be validated by running quality controls at defined intervals and the report signed by the designated/authorised staff with clear writing or printing specifying the measurement procedure with units. The laboratory should not deviate from the standard operating procedure (SOP) which is already prepared by the laboratory for the parameter. There is no room for any errors or corrections in the reporting. Online transfer of results will avoid the transcriptional errors.

20 Sample Rejection

Every laboratory should have clearly spelt out sample rejection criteria and this should be strictly followed. A record should be maintained by the laboratory of the samples that are rejected and the rejected samples should not be sent back to the ward/collection area.

21 Data Management

The laboratory should have a clearly written procedure for reporting results and archiving of data. These records should also have internal and external quality control data, internal audit and calibration data. The archived data should also be stored for a certain number of years which should be specified in the SOP.

22 Standard Operating Procedure

An SOP is a document which contains detailed written instructions describing the stepwise process and technique of performing a test in the lab. The SOP should be strictly adhered to and no deviation should be permitted. The SOP should specify the persons authorised to perform each test, their qualification and training. It also provides safety instruction, trouble shooting, waste disposal, etc. The SOP should be available

to the staff in the working area itself and should be reviewed periodically by competent personnel. There are well-documented formats in which SOPs can be prepared. An SOP should also be prepared for staff training, equipments care, operation and calibration, cleaning, sterilisation and disinfecting procedures, handling and disposal of waste, internal audit, participation in quality control programmes, etc.

23 Laboratory Safety

Since the laboratories use a number of electrical equipments, chemicals and inflammable liquids and gases, the laboratory staff should be well protected from any hazard. The lab will also get large number of infectious materials and it should also protect the staff and the visiting patients from any biohazards. The laboratory's personnel should be aware of all the safety policies and procedures to be followed. The lab should have 'good electrical circuit breakers', 'eyewash facility' and 'fire extinguishers' and there should be also first-aid facilities. Periodic checking of all safety equipment and accessories should be ensured. The laboratory should also appoint a safety officer.

23.1 Biosafety

The laboratory's personnel should be trained on safe hygienic practices like hand washing, wearing protective clothing, gloves and eye protection. Laboratories are classified into four levels of biosafety laboratories. Every laboratory should ascertain to which category they belong to and take appropriate steps to protect the laboratory. The laboratory waste also should be properly segregated according to the biomedical waste rules and should be disposed as per the existing law. The laboratory should also adhere to good ethical practices and there are several principles listed in the ICMR GCLP document which could be followed.

23.2 Quality Assurance

Every laboratory should take measures to give quality results and this can be ensured by participating in quality assurance programmes. There are three stages in the laboratory functioning—pre-analytical, analytical and post-analytical operations. The lab should ensure that in all of these above areas errors do not occur.

The testing laboratories should use the internal quality controls as well as an external quality assurance programme. The quality control samples should be run for both quantitative and qualitative tests. There are several ways of using the data generated by the use of quality control samples, and it is very important to follow some of the quality control guidelines like plotting Levey–Jennings chart, applying Westgard rules or using WHO QC rules. Participating in an EQA scheme is a prerequisite for applying for NABL accreditation. Every laboratory should choose the right quality controls for the improvement of the laboratory.

23.3 Internal Audit

Every laboratory should have a critical review of the functioning and take corrective measures. The audit process could be internal audit, external audit or accreditation. The introduction of new test also should be audited to know whether these new tests are really useful for the patient as well as the clinician.

24 Good Laboratory Practice Guidelines

Scientific measurements (whether they pertain to monitoring contaminants in pharmaceutical products, clinical determinations of blood sugar, characterisation of forensic evidence or testing materials for space missions) are generally recognised as affecting decisions literally concerned with life and death issues. As personal acknowledgement of their responsibility,

scientists have traditionally adopted sound laboratory practices directed at assuring the quality of their data. However, until recently these practices were not consistently adopted, enforced or audited. Because of some notorious historic examples where erroneous data have lead to tragic consequences, national and international agencies have developed guidelines directed at various industries (food, agriculture, pharmaceutical, clinical, environmental, etc.) which fall in the general category of good laboratory practices (GLP). Within the USA, federal agencies such as FDA and EPA have produced documents defining laboratory operational requirements which must be met so that technical data from laboratory studies may be acceptable by those agencies for any legal or contractual purposes. Laboratories doing business with or for these agencies must therefore comply with the specified GLP regulations. So crucial has the issue of maintaining compliance become that many industries report no less than 10%, and occasionally as much as 50%, of their total effort is expanded on internal quality assurance. A typical level of effort is 25%. Since the issue of GLP is very crucial to modern laboratory operations, but most importantly because good laboratory practice is an essential ingredient for any professional scientist, this course will incorporate many of the principles that are part of GLP in contemporary laboratories.

25 Elements of Good Laboratory Practice

25.1 Quality Assurance: Establishing Confidence in Reported Data

The primary products of any laboratory concerned with chemical analysis are the analytical data reported for specimens examined by that laboratory. Quality assurance (QA) for such a laboratory includes all of the activities associated with insuring chemical and physical measurements made properly, interpreted correctly and reported with appropriate estimates of error and confidence levels. Quality Assurance activities also include those maintaining appropriate records of specimen/sample origins and history (sample tracking), as well as procedures, raw data and results associated with each specimen/sample. The various elements of quality assurance are itemised here:

- Standard operating procedures (SOPs)
- Statistical procedures for data evaluation
- Instrumentation validation
- Reagent/materials certification
- Analyst certification
- Lab facilities certification
- Specimen/sample tracking

SOPs are what the name implies, procedures which have been tested and approved for conducting a particular determination. Often, these procedures are evaluated and published by the regulatory agency involved (e.g. EPA or FDA); these agencies may not accept analytical data obtained by other procedures for particular analytes. Within any commercial laboratory, SOPs should either be available or developed to acceptable standards, so that any analytical data collected and reported can be tied to a documented procedure. Presumably, this implies that a given determination can be repeated at any later time, for an identical specimen, using the SOP indicated.

25.2 Statistical Procedures

Many procedural details are optional and arbitrary. Thus, practitioners in a particular field (e.g. agricultural chemistry, clinical chemistry) may adopt certain standards which are deemed acceptable within that field (e.g. using 95 or 99% confidence levels for particular tests), or they may adopt specific statistical analysis procedures for defining detection limits, confidence intervals, analyte measurement units, etc. Regulatory agencies often describe acceptable statistical procedures also.

25.3 Instrumentation Validation

Instrumentation Validation is a process inherently necessary for any analytical laboratory. Data produced by 'faulty' instruments may give the appearance of valid data. These events are particularly difficult to detect with modern computer-controlled systems which remove the analyst from the data collection/instrument control functions. Thus, it is essential that some objective procedures be implemented for continuously assessing the validity of instrumental data. These procedures, when executed on a regular basis, will establish the continuing acceptable operation of laboratory instruments within prescribed specifications. Time-related graphical records of the results of these instrument validation procedures are called 'control charts'. The 'control limits' assigned as upper and lower ranges around the expected instrumental output are generally related to some accepted measure of the random error expected for the overall procedure. (Typically the control limits will be set at ± 2(standard development)) Quality assurance procedures will require that whenever a instrument's performance is outside of the 'control limits', use of that instrument to provide analytical reports must be discontinued; the cause of the problem must be determined and fixed if possible; and the instrument must be certified to be operating again with control limits before returning to service for determinations leading to reported analytical data.

25.4 Reagent/Materials Certification

Reagent/Materials Certification is an obvious element of quality assurance. However, GLP guidelines emphasise that certification must follow accepted procedures and must be adequately documented. Moreover, some guidelines will specify that each container for laboratory reagents/materials must be labelled with information related to its certification value, date and expiration time. This policy is meant to assure that reagents used are as specified in the SOPs.

26 Certification of Analysts

Certification of Analysts is a required part of QA. Some acceptable proof of satisfactory training and/or competence with specific laboratory procedures must be established for each analyst. Because the American Chemical Society does not currently have a policy regarding 'certification' of chemists or analysts, the requirements for 'certification' vary and are usually prescribed by the laboratory in question. These standards would have to be accepted by any agency or client obtaining results from that laboratory. For our student laboratory, the requirement for certification as an analyst is satisfactory completion of the first three laboratory assignments. Execution of these basic procedures will be repeated, if necessary, until satisfactory results are obtained.

27 Certification of Laboratory Facilities

Certification of Laboratory Facilities is normally done by some external agency. For example, an analytical laboratory might be audited by representatives of a federal agency with which they have a contract. An independent laboratory might file documentation with a responsible state or federal agency. The evaluation is concerned with such issues as space (amount, quality and relevance), ventilation, equipment, storage and hygiene. Student chemistry laboratories are generally evaluated by the American Chemical Society, as part of the process of granting approval for the overall chemistry programme presented by the college or university. This latter approval process is not as detailed regarding analytical facilities as the certification processes pursued by agencies concerned specifically with quality assurance.

28 Specimen/Sample Tracking

Specimen/Sample Tracking is an aspect of quality assurance which has received a great deal of attention with the advent of computer-based laboratory information management systems (LIMS). However, whether done by hand with paper files, or by computer with modern barcoding techniques, sample tracking is a crucial part of quality assurance. The terms 'specimen' and 'sample' are often used interchangeably. However, 'specimen' usually refers to an item to be characterised chemically, whereas 'sample' usually refers to a finite portion of the specimen which is taken for analysis. When the specimen is homogeneous (such as a stable solution), the sample represents the overall composition of the specimen. However, for heterogeneous specimens (e.g. metal alloys, rock, soil, textiles, foods, polymer composites, vitamin capsules), a sample may not represent the overall composition. Maintaining the distinction in records of analytical results can be crucial to the interpretation of data.

Procedures for assuring adequate specimen/sample tracking will vary among laboratories. The bottom line, however, is that these procedures must maintain the unmistakable connection between a set of analytical data and the specimen and samples from which they were obtained. In addition, the original source of the specimen/sample(s) must be recorded and likewise unmistakably connected with the set of analytical data. Finally, in many cases the 'chain of custody' must be specified and validated. This is particularly true for forensic samples (related to criminal prosecution) but can also be essential for many other situations as well. For example, a pharmaceutical company developing a new product may be called upon at some time to defend their interpretation of clinical trial tests. Such defence may require the company to establish that specimens collected during these trials could not have been deliberately tampered. That is, they may have to establish an unbroken chain of custody which would remove all doubt regarding the integrity of specimens submitted to chemical analysis.

28.1 Documentation and Maintenance of Records

A central feature of GLP guidelines is the maintenance of records of specimen/sample origin, chain of custody, raw analytical data, processed analytical data, SOPs, instrument validation results, reagent certification results, analyst certification documents, etc. Maintenance of instrument and reagent certification records provides for post-evaluation of results, even after the passage of several years. Maintenance of all records specified provides documentation which may be required in the event of legal challenges due to repercussions of decisions based on the original analytical results. So important is this record-keeping feature of GLP that many vendors are now providing many of these capabilities as part of computer packages for operating modern instruments. For example, many modern computer-based instruments will provide for the indefinite storage of raw analytical data for specific samples in a protected (tamper-proof) environment. They also provide for maintenance of historical records of control chart data establishing the operational quality of instruments during any period during which analytical data have been acquired by that instrument. The length of time over which laboratory records should be maintained will vary with the situation. However, the general guideline followed in regulated laboratories is to maintain records for at least 5 years. In practice, these records are being maintained much longer. The development of higher-density storage devices for digitised data is making this kind of record-keeping possible. The increasing frequency of litigation regarding chemistry-related commercial products is making this kind of record-keeping essential. Moreover, establishing the integrity of the stored data is becoming a high-level security issue for companies concerned about future litigation. All of the ingredients of

record-keeping described above are captured in the scientists' traditional maintenance laboratory notebook.

28.2 Accountability

GLP procedures inherently establish accountability for laboratory results. Analysts, instruments, reagents and analytical methods cannot (and should not) maintain the anonymity that might be associated with a lack of GLP policy. Responsibility for all aspects of the laboratory processes leading to technical results and conclusions is clearly defined and documented. This situation should place appropriate pressure on analysts to conduct studies with adequate care and concern. Moreover, it allows the possibility of identifying more quickly and succinctly the source(s) of error(s) and taking corrective action to maintain acceptable quality of laboratory data.

28.3 GLP for the Chem 55 Laboratory

It is not appropriate to implement a full complement of GLP policies for a student laboratory, as the experimental studies are not related to a commercial product. However, it is useful to incorporate those GLP policies which are fundamental to any sound laboratory work and to provide an introduction to GLP policies that are a part of any contemporary commercial laboratory. Thus, the following GLP policies will be implemented for the Chem 55 course:

- Analyst certification, based on satisfactory performance of basic set of analytical procedures
- Performance of laboratory studies utilising SOPs
- Instrument validation
- Reagent certification
- Laboratory notebook maintenance to contemporary standards
- Maintenance of laboratory records based on instrument and reagent certifications
- Accountability for instrument and reagent certification

29 Good Laboratory Practices for Animal Research

1. These regulations apply to any animal studies for which results will be used to support applications for research or marketing permits for products regulated by the FDA. Such products include human and animal drugs or food additives, medical devices for human use or biological products. GLPs apply to studies aimed at establishing the safety of drugs or devices, not to basic exploratory, mechanism of action or efficacy studies.

   GLPs are complex and require strict adherence for compliance with standards. Detailed standard operating procedures and record-keeping are required for all aspects of the study.

   At a minimum, GLP compliance requires the following:

   - A Study Director, appointed by the institution, who acts as the single source of study control and assures that the protocol is approved and followed, that all experimental data are recorded, that GLPs are followed and that all raw data, documentation, protocols, specimens and final reports are archived as required.
   - An independent quality assurance unit that assures management facilities, personnel, practices and records are in compliance with regulations; maintains a master schedule sheet of studies; inspects each nonclinical study at intervals to assure compliance and reports findings to the Study Director and management; reviews the final report to assure that it accurately reflects the raw data; and prepares and signs a QA statement in the final report.
   - Standard operating procedures for equipment use, maintenance and calibration; laboratory tests and methods; animal use issues such as identification, care, transfer and necropsy; histopathology; handling test and control articles; and data handling and storage. Any deviations from these SOPs must be authorised and recorded by the Study Director.

- A written protocol for each study that describes the objectives and methods for the conduct of the study.
- All data recorded in ink, dated and initialled.
- Separate laboratory and animal facilities.
- Final report containing a compliance statement signed by the applicant, the sponsor and the Study Director.

30 Helpful Resources

The following links will be helpful in further understanding these requirements and when they would be applicable to research.

Having confidence in scientific procedures and data is the sine qua non for determining the safety of chemicals and chemical products. For decisions of safety, there must be rigorous and thorough application of fundamental scientific practices, irrespective of the purpose of the study and where it is conducted—academic, industry or a contract laboratory.

2. Investigations must be designed and conducted by experts; whenever possible, standardised and validated test methods and test systems should be used, test devices and instruments must be appropriately calibrated and their accuracy assured and, most important, all of the data, including raw laboratory records, should be available for independent review. Good laboratory practice (GLP) requirements, based on these fundamental scientific principles and practices, are indispensable for providing scientific confidence in studies conducted for chemical safety determinations. These reasons explain why government agencies worldwide require GLP compliance, and why it is entirely appropriate for greater weight to be given to GLP studies than non-GLP studies that are only available as articles in scientific journals. Noncompliance with GLP should not be used as the sole criterion for excluding studies from consideration in regulatory decision-making. GLP should not be the sole criterion, mischaracterisation of the purpose and function of GLP that GLP has no utility for weighting the reliability of studies.
3. Evaluating the safety of any substance should include review of all relevant studies utilising a systematic weight-of-evidence framework. Although not all studies that are useful for hazard characterisation and risk assessment may be amenable to GLP Tyl (2009) this does not obviate their consideration. Each study, GLP and non-GLP, should be evaluated and weighed in accordance with fundamental scientific principles. Factors to be evaluated include (a) verification of measurement methods and data, (b) control of experimental variables that could affect measurements, (c) corroboration among studies, (d) power (both statistical and biological), (e) universality of the effects in validated test systems using relevant animal strains and appropriate routes of exposure, (f) biological plausibility of results and (g) uniformity among substances with similar attributes and effects. Regulatory agencies and the National Toxicology Program (NTP) require studies to be conducted in accordance with GLP and the Organisation for Economic Co-operation and Development (OECD) GLP principles apply to all OECD member countries (Webster et al. 2005).
4. Academic basic research is very different from regulatory research and testing. Academic research focuses on developing and evaluating new hypotheses, on creating novel methods and on discovering new findings. Academic research is open to wide interpretation and may require significant additional studies to clarify and determine whether and how broadly the results apply. Although novel techniques and discoveries of academic investigations stimulate further research, they must also stand up to the scientific method: hypothesis formulation, hypothesis testing and validation by independent replication. Independent replication provides critical information on the strength of the hypothesis and reliability of test methods. Inconsistent results can arise from use of novel techniques, different test systems, uncertainty and differences in test chemical composition and purity and a myriad

of other factors. These facts, in conjunction with the more limited availability of actual data in most journal publications, mean regulatory agencies can face significant challenges in confirming the quality, performance or data integrity of results obtained solely from information available from a typical article in peer-reviewed journals. Whereas all study records and data from GLP investigations are available to agencies, rarely, if ever, are such details made available as part of the peer-review process for publishing a manuscript in a scientific journal. This can limit the ability of an agency to independently evaluate conclusions or to conduct alternative analyses of the data. The challenges faced by the peer-review procedures of journals have been recently highlighted, and it has been pointed out that '. . .scientists understand that peer review per se provides only a minimal assurance of quality, and that the public conception of peer review as a stamp of authentication is far from the truth'. Journal peer review relies on summarisation of experimental procedures and results and does not include examination of laboratory study records or raw data. The purpose for journal peer review is to judge whether the study has been conducted and reported according to internationally recognised, general scientific standards and whether the study meets the interest level for dissemination to scientific community. It is not designed to provide assurance of accuracy or to recalculate raw data, and it does not provide an opportunity for independent audit of study.

Relevant internationally agreed test methods are used by industry to generate toxicity data for safety determinations by regulatory agencies. Incorporation of GLP in these laboratory tests assures that written protocols and standard operating procedures for each study component are developed carefully and completely followed. GLP also requires meticulous adherence to dosing techniques; the use of adequate group sizes to allow meaningful statistical analysis; characterisation (identity, purity, concentration) of test and control substances, including dosing solutions; detailed recording of study measurements and data; and collection of all raw laboratory data in a manner that can be retained and made available for regulatory agencies to audit and reach independent conclusions. Quality control procedures, quality assurance reviews and facility inspections are also used to monitor and enforce GLP compliance. The relevance, reliability, sensitivity and specificity of most test methods required by industry and regulatory agencies are well understood because they have been subjected to extensive, round-robin validation programmes conducted in numerous laboratories throughout the world. This high level scientific rigour, in conjunction with the detailed processes of GLP, provides regulatory agencies increased confidence in both the relevance and quality of GLP scientific studies for safety decisions, and it is this reason it is wholly appropriate in regulatory decision-making for greater weight and confidence to be afforded to studies conducted in accordance with GLP.

Submission of target animal safety (TAS) data is a requirement for the registration or licensure of veterinary live and inactivated vaccines in the regions participating in the VICH. International harmonisation will minimise the need to perform separate studies for regulatory authorities of different countries. Appropriate international standards will reduce research and development costs by avoiding, when possible, duplication of TAS studies. Animal welfare will benefit because fewer animals will be needed by eliminating repetition of similar studies in each region. This guideline has been developed under the principle of VICH and will provide a unified standard for government regulatory bodies to facilitate the mutual acceptance of TAS data by the relevant authorities. The use of this VICH guideline to support registration of a product for local distribution only is strongly encouraged but is up to the discretion of the local regulatory authority. Furthermore, it is not always necessary

to follow this guideline when there are scientifically justifiable reasons for using alternative approaches.

30.1 Objective

This guideline establishes agreed criteria and recommendations for the conduct of studies that evaluate the safety of final formulation of veterinary live and inactivated vaccines (investigational veterinary vaccines, IVVs) to be marketed for use in target animals.

30.2 Background

The VICH TAS Working Group was formed to develop an internationally harmonised guideline outlining recommendations for meeting regulatory requirements for the registration of IVVs in the regions participating in the initiative. By their nature, guidelines address most but not all possibilities. General principles are included in this guideline to aid in the development of TAS study protocols. It is important to emphasise that the international acceptance of data remains a fundamental principle for VICH.

30.3 Scope

This guideline is intended to cover safety studies of IVVs including genetically engineered products used in the following species: bovine, ovine, caprine, feline, canine, porcine, equine and poultry. This document does not cover TAS studies conducted as part of post-approval batch release requirements. Products for use in minor species or minor uses may be exempted from this requirement for local registration. The guideline will not provide information for the design of TAS studies in other species including aquatic animals. For other species, TAS studies should be designed following national or regional guidance. Additional requirements may apply to genetically engineered products according to the region in which authorisation is sought. Immune modulators are not considered in this guideline. During development, animal safety shall be evaluated in the target animal. The purpose of the evaluation is to determine the safety of the dose of the vaccine proposed for registration. The guideline is therefore limited to the health and welfare of the target animals. It does not include evaluation of food safety or environmental safety including impact on human health.

The guideline is a contribution towards international harmonisation and standardisation of methods used for evaluation of target animal safety of IVVs. The guideline is provided to aid sponsors in preparing protocols for TAS studies conducted under laboratory conditions and in related field studies (which use a larger number of animals). All studies may not be needed. Additional studies not specified in this document and necessary to investigate specific safety concerns of the vaccine in the target animal may be necessary for certain IVVs. Therefore, specific additional information not specified in this document may be determined by communication between the sponsor and the regulatory authority.

31 General Principles

The specific information required to demonstrate target animal safety of an IVV depends upon factors such as proposed usage regimen and dose, type of IVV, nature of adjuvants, excipients, claims, previous use history of similar product, species, class and breed. Generally, the data from safety tests on combined vaccines may be used to demonstrate the safety of vaccines containing fewer antigen and/or adjuvant components provided the remaining components are identical in each case and it is only the number of antigens and/or adjuvant which has decreased. In some regions, this approach may not apply to field safety studies. In this case, each combination of antigens/adjuvant in the final formulation intended to be registered has to be tested. Adverse events must be described and included in the final report and determination of causality for the adverse event attempted.

31.1 Standards

TAS studies done under laboratory conditions should be performed and managed in accordance with the principles of good laboratory practices (GLP), for example, the Organisation for Economic Co-operation and Development (OECD), and field safety studies should be conducted in conformity with the principles of VICH good clinical practices (GCP).

31.2 Animals

The animals should be appropriate for the purpose of the test with regard to species, age and class for which the IVV will be used. Treated and control animals (when used) are managed similarly. The environmental conditions of the groups should be as similar as possible. Housing and husbandry should be adequate for the purpose of the study and conform to local animal welfare regulations. Animals should be appropriately acclimatised to the study conditions. Appropriate prophylactic treatment should be completed before initiation of the study. Reduction or elimination of suffering during the study is essential. Euthanasia and necropsy of moribund animals is recommended.

31.3 IVV and Route of Administration

The IVV and the routes and methods of administration should be appropriate for each type of study.

31.4 Study Design

Where studies performed by a sponsor differ from those specified in this document, the sponsor may conduct a literature search and combine these findings with the results of any preliminary experiments to justify any alternative TAS study designs. Essential parameters to be evaluated for the safety of a vaccine are local and systemic reactions to vaccination, including application site reactions and their resolution and clinical observation of the animals. The reproductive effects of the vaccine shall be evaluated where applicable. Special tests may be required, such as haematology, blood chemistry, necropsy or histological examination. Where these tests are conducted in a subset of animals, these animals should be randomly selected with adequate sampling rate before study initiation to avoid bias, unless otherwise justified. In case of unexpected reactions or results, samples should be selected appropriately in order to identify the cause of the problem observed, if possible. Whenever possible, the personnel collecting data in the studies should be masked (blinded) to treatment identification to minimise bias. Pathologists are not required to be masked to the type of IVV and the possible clinical effects should be masked to the treatment groups. Histopathology data should be evaluated by recognised procedures.

32 Statistical Analysis

In laboratory studies, the safety implications are best addressed by applying descriptive statistical methods to the data. Tables and descriptive text are common methods of data summarisation; however, it may also be valuable to make use of graphical presentations in which patterns of adverse events are displayed both within treatments and within individual animals. In field studies, if applicable, selection of the general form for a statistical model and the factors to be included in the model will depend on the nature of the response variable being analysed and the study design. Regardless of the methods chosen, the process and steps used to conduct any statistical evaluations should be described. The outcomes of the data analysis should be clearly presented to facilitate evaluation of potential safety concerns. The terminology and methods of presentation should be chosen to clarify the results and expedite interpretation.

Although there may be interest in the null hypothesis of no difference between treatment study design constraints limit the statistical power and discriminatory ability of these studies. Under these conditions, statistical analysis alone may not detect potential adverse effects and thus provide assurance of safety. A statistically

significant test does not necessarily indicate the presence of a safety concern. Similarly, a nonsignificant test does not necessarily indicate the absence of a safety concern. Results should therefore be evaluated based on statistical principles but interpretation should be subject to veterinary medical considerations.

32.1 Guidelines

Target animal safety for IVV is determined using laboratory and field studies. For both live and inactivated vaccines, any data collected which could be related to the safety of IVV should be reported from studies conducted during development phase of the IVV. These data may be utilised to support TAS laboratory study design and to identify critical parameters to be examined. Laboratory safety studies are designed to be the first step in evaluating target animal safety and provide basic information before initiating the field studies. The design of laboratory safety studies will vary with the type of product and intended use of the product being tested.

32.2 Laboratory Safety Tests

32.2.1 Overdose Test for Live Vaccines

For live vaccines shown to retain residual pathogenicity by induction of disease-specific signs or lesions, overdose testing of the live vaccine component should be conducted as part of the risk analyses for the acceptability of the microorganism as vaccine strain. The study should be conducted using either a pilot or production batch. A 10X dose based on the maximum release titre for which the application is submitted shall be administered. In the case where the maximum release titre to be licensed is not specified, the study should be conducted with a justifiable multiple of the minimum release titre, taking into account the need to ensure an appropriate safety margin. Exceptions need to be scientifically justified. Generally, eight animals per group should be used unless otherwise justified. If adjuvant or other components are contained in a diluent for the live vaccine, the amount and concentration in a dose administered should be as proposed and justified in the draft registration dossier. If a 10X titre of antigen cannot be dissolved in 1X dose volume, then a double dose or other minimum volume of diluents that are sufficient to achieve dissolution should be used. The inoculum may be administered using multiple injection sites if justified by the required dose volume or the target species.

Generally, for each target species, the most sensitive class, age and sex proposed on the label should be used. Seronegative animals should be used. In cases where seronegative animals are not reasonably available, alternatives should be justified. If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If one route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study. Where applicable, the titre or potency of the batches used for safety testing, particularly the overdose studies, will form the basis for establishing the maximum release titre or potency for batch release.

33 One Dose and Repeat Dose Test

For vaccines that require a single lifetime dose or primary vaccination series only, the primary vaccination regimen should be used. For vaccines that require a single dose or primary vaccination series followed by booster vaccination, the primary vaccination regimen plus an additional dose should be used. For convenience, the recommended intervals between administration may be shortened to an interval of at least 14 days. Evaluation of the one/repeat dose testing should be conducted using either a pilot or production batch of IVV containing the maximum release potency; in the case where maximum release potency to be licensed is not specified, then a justified multiple of the minimum release potency should be used. Generally, eight animals per group should be used unless otherwise justified. Generally, for each target species, the most sensitive class, age and sex proposed on the

label should be used. Seronegative animals should be used for live vaccines. In cases where seronegative animals are not reasonably available, alternatives should be justified. If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If one route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study.

33.1 Data Collection

General clinical observations appropriate for the type of IVV and animal species should be made every day for 14 days after each administration. In addition, other relevant criteria such as rectal temperature (for mammals) or performance measurement are recorded within this observation period with appropriate frequency. All observations should be recorded for the entire period. Injection sites should be examined daily or at other justified intervals by inspection and palpation for a minimum of 14 days after each administration of the IVV. When at the injection site adverse reactions are present (at the end of the 14-day observation), the observation period should be extended until clinically acceptable resolution of the lesion has occurred or, if appropriate, until the animal is euthanised and histopathological examination is performed.

33.2 Reproductive Safety Test

Examinations of reproductive performance of breeding animals must be considered when data suggest that the starting material from which the product is derived may be a risk factor. The laboratory studies in concert with the field safety studies are required to support use in breeding animals. If the reproductive safety studies are not performed, an exclusion statement must be included on the label, unless a scientific justification for absence of risk for use of the IVV in the breeding animal is provided. The design and extent of the laboratory and field safety studies will be based upon the type of organism(s) involved, the type of vaccine, timing and route of delivery and the animal species involved.

For examination of reproductive safety, animals appropriate for the purpose of the study will be vaccinated with at least the recommended dose according to the vaccination scheme indicated. If multiple routes and methods of administration are specified for the product concerned, administration by all routes is recommended. If one route of administration has been shown to cause the most severe effects, this single route may be selected as the only one for use in the study. Generally, 8 animals per group should be used unless otherwise justified using either a pilot or production batch. The animals should be observed for a period appropriate to determine reproductive safety, including daily safety observations. Exceptions should be justified. A control group should be included.

Vaccines recommended for use in pregnant animals must be tested as described above in each of the specific periods of gestation recommended for use in the label. An exclusion statement will be required for those gestation periods not tested. The observation period must be extended to parturition, to examine any harmful effects during gestation or on progeny. Exceptions should be justified. When scientifically warranted, additional studies may be required to determine the effect(s) of IVV on semen, including shedding of the live organism in semen. The observation period should be appropriate for the purpose of the study. For IVVs recommended for use in future layers or laying hens, study design should include evaluation of parameters that are appropriate for the class of hens vaccinated.

33.3 Field Safety Test

Where disease and husbandry are similar between regions participating in the VICH, international data may be used for field studies, as long as a minimum proportion of the data, acceptable to the regional authorities, is generated within the region where approval is being sought. It is the responsibility of the sponsor to ensure that field studies should be conducted under animal husbandry

conditions representative of those regions in which authorisation is sought. Local authorisations must be obtained prior to conduction of the study. Consultation with regional regulatory authorities regarding study design prior to conduction of the studies is recommended. If a label indicates use in breeding animals, appropriate field safety studies need to be performed to show the safety of the IVV under field conditions.

34 Animals

The animals should be in the age range/class intended for treatment as indicated in the proposed labelling. Serological status may be considered. Whenever possible either a negative or positive control group is included. Treated and control animals are managed similarly. Housing and husbandry should be adequate for the purpose of the study and conform to local animal welfare regulations.

34.1 Study Sites and Treatment

Two or more different geographical sites are recommended. The recommended dosage(s) and route(s) for vaccination should be used. The studies should be conducted using representative batch(es) of the IVV. Some regions may require that the field safety study be performed using more than one batch of product.

34.2 Data Collection

Observations should be made over a period of time appropriate for the IVV and adverse events should be documented and included in the final report. Reasonable attempts should be made to determine causality for the adverse event. Submission of target animal safety (TAS) data is a requirement for the registration or licensure of veterinary live and inactivated vaccines in the regions participating in the VICH. International harmonisation will minimise the need to perform separate studies for regulatory authorities of different countries. Appropriate international standards will reduce research and development costs by avoiding, when possible, duplication of TAS studies. Animal welfare will benefit because fewer animals will be needed by eliminating repetition of similar studies in each region.

This guideline has been developed under the principle of VICH and will provide a unified standard for government regulatory bodies to facilitate the mutual acceptance of TAS data by the relevant authorities. The use of this VICH guideline to support registration of a product for local distribution only is strongly encouraged but is up to the discretion of the local regulatory authority. Furthermore, it is not always necessary to follow this guideline when there are scientifically justifiable reasons for using alternative approaches.

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