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FRESHWATER QUALITY MONITORING WITH BIOTA

Technical Guidance Document

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ABOUT THIS GUIDEBOOK

This technical guidance document is intended for scientists and practitioners who work with freshwaters in the field and in the laboratory but who do not have specialist knowledge of ecology and the use of biota for water quality monitoring. It introduces some fundamental aspects of freshwater ecosystems and ways in which human activities can affect them. Understanding impacts on biota and ecosystems can guide development and selection of approaches to using freshwater biota to monitor water quality. Successful monitoring with biota depends on using appropriate sampling protocols and related data analysis. Some of the more common approaches suitable for rivers, lakes and reservoirs are described in detail to illustrate their use, together with selected options and examples for analysing data derived from these methods. General data analysis techniques are covered in the companion guidebook in this series which covers data handling and assessment techniques for ambient water quality monitoring data: "*Water Quality Data Handling and Assessment*". Although most microbiological monitoring is done to ensure water is safe to drink, it is described briefly here as a tool for determining the presence of faecal contamination in surface and groundwaters. A range of widely-used field sampling methods for the major groups of biota are also illustrated.

The information provided here will assist water resource managers in deciding whether to incorporate the use of biota in their water quality monitoring programmes to support management action and policy development towards sustainable use of freshwater resources.

It is strongly recommended that this guidebook is read in conjunction with the accompanying guidebooks in the series, particularly "*An Introduction to Freshwater Quality Monitoring and Assessment*", "*Water Quality Monitoring and Assessment in Rivers*, *Lakes and Reservoirs*" and "*Quality Assurance for Freshwater Quality Monitoring*".

The complete series of guidance documents that address various aspects of monitoring and assessment of freshwater are:

- Introduction to Freshwater Quality Monitoring and Assessment
- Water Quality Monitoring and Assessment of Groundwater
- Quality Assurance for Freshwater Quality Monitoring
- Water Quality Monitoring and Assessment in Rivers, Lakes and Reservoirs
- Freshwater Quality Monitoring with Biota
- Freshwater Quality Monitoring using Particulate Matter
- Water Quality Data Handling and Assessment

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
ASPT	Average Score Per Taxon
ATP	adenosine triphosphate
BMWP	Biological Monitoring Working Party score
CEN	European Committee for Standardization
CFUs	Colony Forming Units
DES	Department of Environment and Science
DNA	deoxyribonucleic acid
DO	dissolved oxygen
EC	European Commission
EC	effective concentration
EEA	European Environment Agency
EPA	Environmental Protection Agency
EU	European Union
FWQMS	Freshwater Quality Monitoring and Surveillance
GEMS	Global Environment Monitoring System
IBI	Index of Biological Integrity
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry

ISMR	Saprobic Index for Minas Gerais and Rio de Janeiro states
ISO	International Organization for Standardization
LC	lethal concentration
MATC	Maximum Allowable Toxic Concentrations
MPN	most probable number
MUG	4-methylumbelliferyl-β-glucuronide
OECD	Organisation for Economic Co-operation and Development
ONPG	ortho-nitrophenyl-β-D-galactopyranoside
PCBs	polychlorinated biphenyls
REMP	River Eco-status Monitoring Programme
UNEP	United Nations Environment Programme
USEPA	US Environmental Protection Agency
USGS	United States Geological Survey
VTEC	Verotoxigenic-producing Escherichia coli
WAP Rhine	International Warning and Alarm Plan for the River Rhine
WHO	World Health Organization
WHPT	Whalley Hawkes Paisley and Trigg metric

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Chapter 1

INTRODUCTION

Traditionally, water quality has been monitored using physical and chemical parameters, such as pH, electrical conductivity, nitrate, and heavy metals, by making *in situ* measurements with sensors or by taking grab samples and analysing them in the laboratory. Grab samples provide a measure of water quality at the instant and location of collection for individual water quality parameters. They can indicate changes or differences in physical and chemical water quality temporally and spatially but do not show how such changes actually affect the aquatic environment and the biota living there. Changes in aquatic biota over time reflect the integrated impacts (physical and chemical) of all pressures on the water body. Even where specific impacts on biota from measured compounds might be expected, such as for toxic organic contaminants, the measurements of individual compounds give no information about how several compounds might interact chemically within the environment, and whether such interactions result in different impacts on the biota. Although it has been widely acknowledged for decades (Organisation for Economic Co-operation and Development [OECD] 1987) that the biota themselves can provide information on collective impacts on water quality from human activities, the concept of incorporating them into regular monitoring and assessments of water quality did not gain momentum until the European Union (EU) implemented the Water Framework Directive in 2000 (European Commission [EC] 2000) requiring member countries to reach both good chemical and good ecological status (EC 2005). Standardising such an approach across all EU member States (EC 2014) has been challenging with different EU countries taking different approaches (European Environment Agency [EEA] 2016). Nevertheless, there are now many countries and several river basin organisations worldwide that have incorporated elements of biological assessment into their water quality monitoring programmes, such as the River Eco-status Monitoring Programme (REMP) of South Africa (https://www.dws.gov.za/iwqs/rhp/default.aspx), the Mekong River Commission's Ecological Health Monitoring activities (https://www.mrcmekong.org/our-work/functions/basin-monitoring/ecological-health-monitoring/) and the Freshwater Quality Monitoring and Surveillance (FWQMS) programme of Canada (https://www.canada.ca/en/environment-climate-change/services/freshwater-quality-monitoring/overview.html#cabin).

It is not possible in this guidebook to describe all the different methods for monitoring water quality using biota. Methods are constantly being revised and new methods are being developed. In addition, some methods are only suitable for local use. Hence, this guidebook focusses on introducing the main concepts and approaches to monitoring freshwater quality with biota. It concentrates on the approaches that are applicable worldwide and are suitable for inclusion in routine water quality monitoring programmes for determining human impacts on freshwater quality and the associated damage to freshwater ecosystems.

Selecting and applying the appropriate monitoring method using biota requires an understanding of the freshwater ecosystem for which it will be used, the potential impacts that are to be assessed and an appreciation of the strengths and weaknesses of the available methods (see sections 1.1 and 1.2).

Such methods are commonly used alongside, and complementary to, other physical and chemical monitoring and assessment approaches for freshwaters. The chosen method should provide data that will meet the needs of the monitoring programme objectives (see the accompanying guidebook on "*An Introduction to Freshwater Quality Monitoring and Assessment*").

BOX 1.1 TERMINOLOGY RELATING TO FRESHWATER ECOSYSTEMS

In an ecosystem, individuals of a single *species* form *populations*. Populations of two or more species interact with each other and their environment to form an ecological *community*. The species of plants and animals that are typically together in an area with characteristic environmental factors like substrate, light, oxygen, temperature, and organic matter, are known as biological communities. These plants and animals have similar preferences for, and tolerances of, those environmental factors.

A species distribution comprises the different geographic locations in which populations of the species occur; this is referred to as the *species range*.

Biomass is the total living (or recently living) biological matter at a given time. For example, species biomass refers to the mass of a specific species, whereas community biomass can include all the species of the community, such as microorganisms, plants and animals.

A food chain begins with a *primary producer* and ends with a top consumer. Primary producers, also known as autotrophs, are the primary source of biomass (e.g., phytoplankton, algae and macrophytes). They are the first trophic level (steps of the food chain) in every ecosystem. Animals that depend on primary producers as a food source are known as primary consumers. Primary consumers, such as zooplankton, that principally consume algae and plants are herbivores. Typically, carnivores and omnivores, such as fish, feed on the primary consumers. Decomposer organisms (microbes and detritivores), such as bacteria and fungi, are important for recycling organic matter and nutrients back into the food chain.

The biology and ecology of freshwater bodies vary globally and from water body to water body, with the flora and fauna influenced by hydrological, physical and chemical factors that may be different in each water body. An understanding of hydrobiology is, therefore, fundamental to the successful implementation of biological monitoring approaches, and particularly to the interpretation of the monitoring data obtained. Some common terminology used in relation to monitoring with biota is given in **Box 1.1**. A brief introduction to the functioning of ecosystems, together with the general principles for establishing and conducting monitoring programmes for water quality in rivers, lakes and reservoirs are available in the accompanying guidebook "*Water Quality Monitoring and Assessment in Rivers, Lakes and Reservoirs*".

1.1 Impacts on aquatic communities

Both natural processes and anthropogenic activities can disturb freshwater ecosystems and lead to changes in freshwater communities. Common disturbances include: organic matter pollution leading to dissolved oxygen depletion; increased suspended solids and sediment deposition; habitat modifications such as river embankments and dredging; changes in hydrological regime due to weirs and dams; and pollution or contamination with natural (e.g., excess nutrients) or synthetic (e.g., pesticides) compounds. Most organisms living in a water body are sensitive to changes in their environment, and these disturbances can result in the death or migration of individuals which subsequently affects the populations of species present. Less obvious responses to disturbances can include changes in metabolism and reduced reproductive capacity, which affect species and population growth. Responses to disturbance can be used in biological monitoring of the state of freshwater systems, detection of contaminants, or in understanding the effects of multiple stressors on aquatic ecosystems.

1.1.1 Physical and chemical changes

Changes in dissolved oxygen (DO) concentrations affect aquatic biota directly and indirectly. Such changes can occur naturally, such as at the bottom of lakes as a result of decomposition of dead phytoplankton that have settled from the water column. Anthropogenic activities, such as sewage discharges, milk spillages, thermal pollution, and excess nutrient run-off leading to eutrophication can result in decreases in dissolved oxygen concentrations. The decline in DO in the water causes stress for aquatic organisms and can result in serious disruption of the ecosystem, including fish-kills, if the organisms are not able to seek refuge in unaffected or less affected areas. Warm water holds less dissolved oxygen than cold water, thus discharges of wastewater that are above the natural temperature of an aquatic system can also affect dissolved oxygen concentrations. When organisms are subjected to thermal stress their growth and development rate, number of life cycles per year, body size, and competitiveness may be negatively affected (Alfonso, Gesto and Sadoul 2021; Bonacina *et al.* 2023). Some species have a greater tolerance for changes in temperature or have a higher thermal threshold before experiencing stress. Salmonid fish, for example, are usually restricted to living in temperatures lower than 25°C, while Cyprinids such as Carp can tolerate <30–35°C.

Eutrophication (nutrient enrichment) can cause an increased growth of algae and macrophytes that reduce oxygen concentrations in the water when they die and decompose. Hence, species that are tolerant of reduced oxygen concentrations can be dominant in eutrophic water bodies. The degree of tolerance to reduced dissolved oxygen forms the basis of some biotic indices and water quality assessment methods that are discussed in chapter 2.

Some natural events, such as volcanic eruptions, and most human activities have the potential to release toxic elements and compounds to the aquatic environment via atmospheric deposition, wastewater discharges and terrestrial run-off. Toxic compounds have the ability to cause damage to an organism through interference with normal physiological processes, such as growth and reproduction and, in the case of acute toxicity, they can cause rapid death. Even natural compounds that are essential for life in trace or small amounts, such as copper (Cu) and zinc (Zn), can become toxic when in excess; see, for example, Shahjahan *et al.* (2022) for a review of the effects in fish.

A water body may receive many different contaminants from different sources, and the combination of these contaminants may vary both spatially and temporally. To determine the impacts of contamination in water bodies on their biota, it is often preferable to study the biota themselves. In addition, studying the organisms *in situ* allows the impact of exposure to several different (perhaps unknown) contaminants or toxins to be evaluated, especially where synergistic effects are unknown or not well understood. Different approaches to using biota to monitor the presence and impact of toxic contaminants are presented in chapter 3.

Changes in pH in aquatic ecosystems can influence the bioavailability and uptake of contaminants by aquatic organisms. Some natural compounds, such as metals, can become toxic to organisms when adsorbed to high levels within their bodies. Regions where water bodies naturally have low buffering capacities (i.e., low water hardness/ low concentrations of carbonates) are susceptible to acidification of the water bodies. Acidification increases the solubility and mobility of toxic metals such as cadmium and mercury, which can then bioaccumulate in the tissues of aquatic organisms such as fish (see chapter 3), causing a potential health risk to women and men who consume them. Reduced pH can also be a source of stress to sensitive organisms, and even lethal if the pH drops below the tolerance limit of a species.

1.1.2 Hydromorphological disturbance

Hydromorphological disturbances, either natural (e.g., rainstorms or droughts) or caused by human activities (e.g., damming, canalisation and drainage of rivers), often cause changes in flow and substrate which can result in temporary or permanent loss of species in aquatic systems. Events, such as high or extreme rainfall, result in run-off carrying additional sediment and organic matter into water bodies. The

flow rate of a river can also increase greatly, causing the settled sediments at the bottom of the channel to be brought back into suspension and to be carried downstream, disturbing benthic habitats. This, in turn, affects the richness and abundance of organisms, such as macroinvertebrates, in the river (Feeley *et al.* 2012). High flows can also cause small organisms, such as macroinvertebrates, to be detached from their habitats on the bottom of the channel or amongst the macrophytes, and to be carried downstream. Intense rainfall, leading to increased discharge in rivers, can result in physical damage and abrasion of the banks and river bed, changing the habitats within the streams. The extent to which physical disturbance affects the water body can be described, therefore, by the presence, absence and abundance of organisms in the different habitats.

Increases in suspended particulate matter caused by natural or anthropogenic events such as dredging and construction of embankments (**Fig. 1.1**) hinder light penetration in the water column and reduce food and oxygen supply to benthic biota that become buried as the particulate matter settles. Some benthic organisms, such as worms, may be able to adapt to this smothering by migrating vertically upwards through the deposited sediments. However, immobile organisms, such as attached mussels, often die and take some time to recover their former population levels. The impact of siltation on benthic communities is determined by the thickness of the deposits and the intensity and frequency of deposition events. For example, the slow addition of thin layers of sediment is tolerated better than the same thickness of sediment deposited in a single event. Increased turbidity can also reduce the respiratory functions of some organisms as well as their ability to find food or avoid predators. Changes in the composition of the substrate, e.g., from sand, gravel, or rock to mud or silt, can reduce juvenile fish survival, eliminate or modify spawning habitats, and impact the overall benthic community diversity. A review of the impacts of suspended solids and turbidity on aquatic biota is available by Bilotta and Brazier (2008).

Figure 1.1 **Flood protection works on the Bandon River, Cork, Ireland creating major hydromorphological alterations to the river – destroying benthic habitats and increasing suspended solids. © Patrick Cross**

1.1.3 Domestic wastewater

Domestic wastewaters contain organic matter and nutrients (mainly nitrogen and phosphorus), and often a wide range of other contaminants such as trace metals, pharmaceutical residues, or synthetic compounds arising from use of personal care products (Margot *et al.* 2015). When industrial effluents are combined with domestic sewage, the resultant effluent may contain other contaminants which may be toxic to living organisms (e.g., chlorinated hydrocarbons). Hynes (1960) described the pattern of changes in water quality and aquatic organisms in a river following the continuous discharge of domestic sewage. These classic changes have subsequently aided the development of monitoring approaches based on biota. Following the classically described pattern, there is usually a decline in DO concentrations at the discharge point and immediately downstream and salts and suspended solids concentrations increase but gradually decline with distance downstream as the salts are diluted and the suspended solids settle to the river bed. Other measurable effects include the increase of ammonium (NH_4^+) downstream from the discharge. As DO increases, the ammonium decreases and

nitrate increases because bacteria convert ammonium to ammonia (NH_3) and to nitrate in the presence of oxygen. If the pH goes above 8.5, levels of NH_3 increase, which is highly toxic to fish populations.

Some organisms that are adapted to survive low dissolved oxygen concentrations exploit the increase in organic matter and associated nutrients coming from the sewage discharge. Sewage fungus (a community of mixed microorganisms that are tolerant of low oxygen) and bacteria populations increase rapidly downstream of the discharge and return to near previous levels downstream as nutrients are depleted. Algal populations may be reduced close to the discharge due to the increased turbidity but may take advantage of the increased nutrients slightly downstream, especially the filamentous green alga, *Cladophora*.

Tubificidae (also known as Naididae) are worms that are tolerant of low oxygen concentrations and are able to take advantage of the increased organic matter immediately downstream of the sewage discharge. As oxygen concentrations increase downstream, *Chironomus* (non-biting midge) populations rise rapidly and Tubificidae populations decline. *Asellus* populations (a crustacean that consumes detritus) increase rapidly when oxygen concentrations begin to increase and there is an abundant food source in the form of algal material and detritus.

1.2 Deciding whether to include biota in a water quality monitoring programme

Including biota in a monitoring programme can demonstrate long-term change in water quality status as a result of natural and anthropogenic impacts in a water body and can assist with determining the spatial scale of impacts such as a) distance downstream affected by a point source pollutant, b) proportion/area of a lake affected by a point source pollutant, and c) geographical spread of the influence of an atmospheric pollutant on lakes or rivers (see chapter 2). Biota can also be used to examine the toxic effects of contaminants on individual organisms and communities for compliance or early warning monitoring (see chapter 3) and to determine the potential for different health risks for women, men and children consuming aquatic biota that have bioaccumulated toxic substances; particularly risks for pregnant women and very young children who may be more susceptible than the general population. Monitoring the presence and abundance of pathogenic microorganisms in the water (see chapter 4) can also help to protect human health.

When considering whether biota could be useful for meeting the objectives of a freshwater quality monitoring programme, the relative cost, ease-of-use, and provision of complementary information to other monitoring techniques, like physicochemical analysis, should be assessed. It is also essential to be aware of the limitations and potential advantages of the different approaches (**Table 1.1**). A selection of reasons why biota might be included in a freshwater quality monitoring programme are given in **Box 1.2**.

BOX 1.2 EXAMPLES OF REASONS FOR INCLUDING BIOTA IN A FRESHWATER QUALITY MONITORING PROGRAMME

- To determine status and trends in water quality and ecosystem health.
- To evaluate the transport and distribution of contaminants in the aquatic environment.
- To determine the potential risks to an aquatic ecosystem from the presence of contaminants in different organisms.
- To protect human health by measuring the concentrations of contaminants in aquatic organisms that are used as a food source, e.g., fish and shellfish.
- To establish the presence of contaminants where the concentrations in water samples may be below the limits of detection for the analytical methods available.

Table 1.1 **Relative limitations and advantages of physical and chemical monitoring approaches compared with the use of biota for monitoring**

Monitoring potential	Relative performance	
	Physical/Chemical	Biota
Precision (i.e., assessment of pollutant concentration)	Good	Poor
Discrimination (what type of pollution)	Good	Poor
Representativeness (how likely is it that a limited number of samples truly reflect system status)	Poor	Good
Measure of effects	No	Yes
Relative costs (e.g., need for ecological or taxonomic expertise, requirements of the specific approach, availability and sensitivity of analytical methods)	High	Low

Only a limited number of the observed effects of human activities on species and communities in freshwaters have been developed into monitoring methods that are nationally, regionally, or globally applicable. Even where methods could be applied across different geographical areas, there is often a need to validate and adapt those methods for local use. This usually requires the assistance of scientists who have specialist knowledge about aquatic organisms and communities in the area or region of interest. It is important therefore to ascertain whether suitable methods exist that will contribute to the monitoring programme objectives and whether financial and personnel resources will be needed to test, validate, and possibly modify the method. It is also important to check whether the response rate and sensitivity of the biological process on which the monitoring method is based matches the degree and duration of the anticipated impact on the waterbody to be monitored. The principal approaches to monitoring using biota are given in **Box 1.3** and discussed in chapters 2 and 3.

BOX 1.3 PRINCIPAL APPROACHES TO MONITORING FRESHWATER QUALITY USING BIOTA

Ecological methods based on the impacts on species populations and on the structure of the aquatic community.

Physiological and biochemical methods that measure aspects of the metabolism within a community (e.g., oxygen consumption), or within individuals (e.g., enzyme inhibition).

Histological and morphological methods based on observations of changes in organisms such as skin tumours in fish, or gill damage.

Controlled biotests which examine the effects on individuals such as growth, reproductive ability, death.

Chemical analysis of contaminants accumulated in the tissues of aquatic species, i.e., bioaccumulation.

Microorganisms are often included in water quality monitoring programmes specifically to assess risk to human health from pathogenic organisms that are excreted and transported to water bodies with wastewaters or run-off. Some of the organisms commonly monitored, e.g., faecal coliform bacteria, are not aquatic and have limited ability to survive in freshwater environments. Some others, e.g., *Giardia* and *Cryptosporidium*, use water as a means of transmission to a new host and are difficult to monitor on a routine basis. The detailed methods for monitoring microorganisms are beyond the scope of this guidebook but the principal approaches that are useful for water quality monitoring programmes are described in chapter 4.

Chapter 2

ECOLOGICAL METHODS

Ecological approaches are normally used to monitor status and trends in the quality of surface waters or to compare different locations within a water body. They can also be used to monitor impacts on the ecosystem from human activities. There have been many methods developed for different purposes and for use in a variety of situations and geographic regions. It is beyond the scope of this guidebook to describe methods in detail and, therefore, this chapter describes some of the principles on which most methods are based and illustrates how they have been applied with a few examples.

Ecological methods can be broadly divided into indicator species methods and community-based methods. There are several factors to consider when deciding which approach is best suited to the objectives of the monitoring programme. These are summarised in **Table 2.1**.

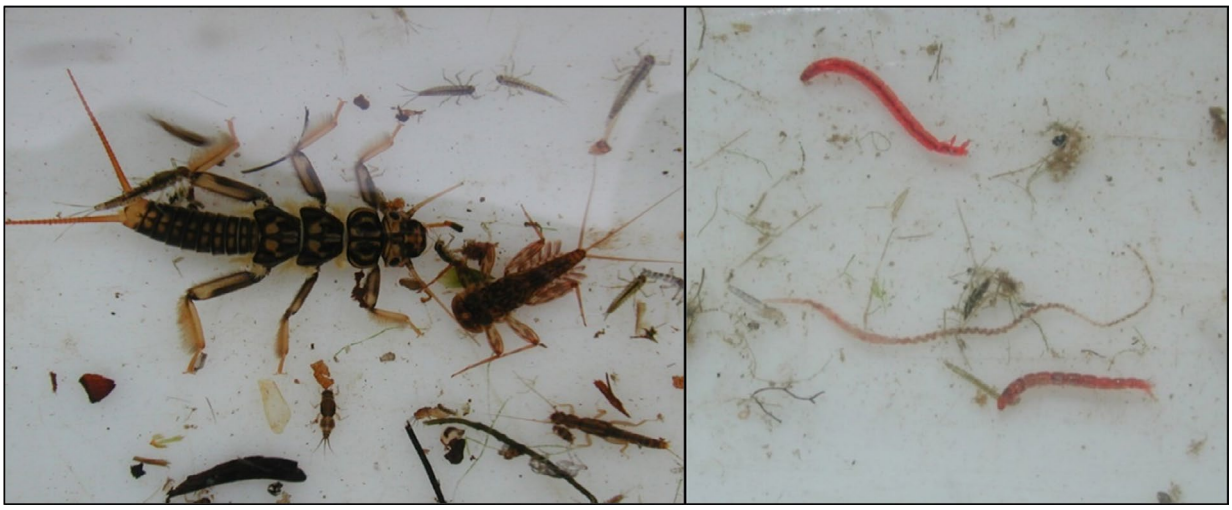
2.1 Indicator species approach

The environmental requirements or tolerances, such as high or low oxygen concentrations, of individual species determine their presence or absence within the aquatic ecosystem. Their degree of sensitivity may indicate the level of pollution in a habitat (**Fig. 2.1**). Species with specific environmental tolerances are often referred to as *indicator organisms* or *bioindicators*. Indicator species have been incorporated into methods that are used extensively for water quality monitoring, especially in rivers. These methods often provide a non-specific indication of anthropogenic impacts on the waterbody.

Table 2.1 Features of indicator species and community-based ecological monitoring approaches

Indicator species	Communities of species
High natural temporal and spatial variance	Lower natural temporal and spatial variance
Require quantitative sampling	Semi-quantitative or qualitative sampling required
Narrow range of tolerance to change in water quality	Wide range of tolerance to change in water quality
Rapid and consistent response to a particular pollutant	Show general effects for many potential pollutants and combinations of pollutants

Figure 2.1 **Typical macroinvertebrates found in good (left) and poor (right) water quality in rivers and streams. The organisms on the left are stonefly and mayfly nymphs. The chironomid larvae and tubificid worm on the right are tolerant of low oxygen concentrations. © Patrick Cross**



To use individual species as indicators of water quality they should have the following characteristics:

- A narrow ecological range (i.e., they are specialists rather than generalists).
- Reliable identification using routine laboratory equipment.
- Well defined taxonomy.
- Wide geographic distribution.
- A relatively rapid response to environmental changes.

The potential suitability of the different groups of aquatic organisms as indicators of water quality is given in **Table 2.2**.

Of the groups shown in Table 2.2, benthic macroinvertebrates are the most widely used aquatic organisms for ecological monitoring because:

- They are widely distributed globally in almost all types of water body.
- There are similar species and communities that can be found in similar water bodies globally.
- They are easy and generally cheap to sample.
- Their lifespan is long enough to be able to reflect long-term pollution impacts.
- They indicate the local conditions of their habitat because of their relatively sedentary lifestyle (i.e., migration away from pollution is often not possible).

The most common approach to using indicator species is to assign a score to individual species, or groups of species, based on their tolerance to the disturbance being monitored, such as organic matter pollution. Simple *biotic indices* combine the scores for the species identified in a sample into a single numeric value. When sufficient background knowledge exists about the tolerances of the local species, the score can indicate the degree of disturbance for the sample site, and the range of potential index values represents water quality from poor to excellent. Biotic indices are very useful for communicating water quality to a general audience because a simple numerical scale can easily be understood. This approach is particularly useful for spatial monitoring as demonstrated by Sinche *et al.* (2022) for the Orienco stream in Northern Equador and long-term trend monitoring, for example in streams in the Delaware River basin in the USA (United States Geological Survey [USGS] 2002).

Table 2.2 Advantages and disadvantages of different groups of organisms as indicators of water quality

Organism group	Advantages	Disadvantages
Bacteria	Routine methodology well developed. Rapid response to changes, including pollution. Some species are indicators of faecal pollution. Relative ease of sampling.	Cells may not have originated from sampling point. Populations fluctuate rapidly with intermittent pollution. Special equipment necessary for some species.
Protozoa	Known preferences for levels of organic matter in water. Rapid responses to changes in water quality. Relative ease of sampling	Good taxonomic expertise required. Cells may not have originated from sampling point. Indicator species may also occur in unpolluted water bodies.
Algae	Pollution tolerances well documented for some species. Useful indicators of eutrophication and increased turbidity.	Taxonomic expertise required. Rarely useful for severe organic or faecal pollution. Sampling and enumeration can be difficult for some species and groups.
Macroinvertebrates	Diverse morphologies and lifestyles, hence found in many different habitats. Many sedentary species can indicate effects at the site of sampling. Whole communities can respond to change. Long-lived species can indicate integrated pollution effects over time. Qualitative sampling is easy. Sampling equipment is simple and inexpensive. Good taxonomic keys available.	Quantitative sampling is difficult. Substrate type is important when sampling. Species may become dislodged and drift in fast moving waters. Knowledge of life cycles necessary to interpret absence of species. Some groups difficult to identify to species level.
Macrophytes	Usually attached to the substrate. Easy to see and identify. Good indicators of suspended solids and nutrient enrichment.	Responses to pollution not well documented for most species. Often tolerant of intermittent pollution. Many occur seasonally.
Fish	Sampling and enumeration methods well developed. Immediate physiological responses to pollution and contaminants can be obvious. Can demonstrate food chain effects. Relative ease of identification.	Species may migrate to avoid pollution.

Source: Based on Hellawell (1977)

Biotic indices are based on the presence or absence of indicator species that are sensitive to, or tolerant of, a specific form of water quality degradation, such as low oxygen concentrations or acidity. Due to natural variations in species distributions, most biotic indices are either developed for local aquatic ecosystems or validated for local use. To create or validate a biotic index expert knowledge is required of habitat preferences, environmental tolerance ranges, and sensitivities to the pollutants of interest. Considerable taxonomic expertise may also be required to identify the species reliably at all life cycle stages. Although biotic indices are very informative, they should be used together with other water quality data to interpret the biotic index correctly.

There are many biotic indices that have been developed to monitor water quality in specific countries and regions. Examples using diatoms (unicellular microalgae) are described by Cost *et al.* (2009), Lavoie *et al.* (2014), Lobo *et al.* (2016) and Masouras *et al.* (2021). Examples using macroinvertebrates, include the Whalley Hawkes Paisley and Trigg (WHPT) metric for assessing river invertebrate communities in the United Kingdom of Great Britain and Northern Ireland (UK) (Walley and Hawkes 1996; Walley and Hawkes 1997; Paisley *et al.* 2007), the Irish Environmental Protection Agency (EPA) Q-value (Toner *et al.* 2005) and the Saprobic Index for Brazilian Rivers in Minas Gerais and Rio de Janeiro states (ISMR) described by Junqueira *et al.* (2010).

2.1.1 The Biological Monitoring Working Party score

One of the most well-known biotic indices for freshwaters is the Biological Monitoring Working Party (BMWP) score, which was developed originally in the UK (Hawkes 1998) and has been adapted for use in countries worldwide because it is based on identifying benthic macroinvertebrates only to the family level, instead of the more labour-intensive identification to species level (Hawkes 1977). It is widely applicable because it is not specific to any single river catchment or geographical region. The indicator organisms are particularly sensitive to decreases in oxygen concentrations associated with organic pollution. Therefore, the score system is mostly used to determine the impact of pollution events, particularly organic pollution arising from agricultural activities or sewage. The macroinvertebrates are identified to family level and then each family is given a score between one and ten (**Table 2.3**). The closer to ten, the less tolerant the organism is to pollution and its presence indicates clean water. A score of ten represents the most sensitive organisms (e.g., stonefly and mayfly nymphs – Fig. 2.1). Molluscs are only mildly sensitive to organic pollution and therefore score three, whereas pollution-tolerant worms (e.g., Oligochaeta) score one. The overall BMWP score is calculated by summing all the tolerance scores of the macroinvertebrate families found in the sample, with higher scores reflecting better water quality. Advice for the use of benthic macroinvertebrates for the biological classification of rivers is available in International Organization for Standardization (ISO) standard ISO 8689-1:2000 (ISO 2000) and there are numerous macroinvertebrate identification guides available, such as Croft (1986), Smith, Storey and Valois (2019) and the on-line atlas and identification key for macroinvertebrates of Easter North America (https://www.macroinvertebrates.org/).

The number of different families recorded in a sample is also noted because it gives an idea of the diversity of the community and is used in calculating the Average Score Per Taxon (ASPT). Less disturbed or unpolluted water bodies usually have a high diversity of organisms. The ASPT is the average sensitivity of the families of the organisms in the sample and is calculated by dividing the BMWP score by the number of families found in the sample. Comparing the ASPT between samples reduces the effects of sample size, sampling effort and sampling efficiency on the results because the ASPT is less sensitive to sample effort and natural differences in diversity between streams. An example of good water quality using the BMWP score and ASPT, is a BMWP score of higher than 100 with an ASPT of greater than 4. The procedure for assessing water quality with the BMWP score is described in **Box 2.1**.

Table 2.3 An example of scores allocated to invertebrate families based on the BMWP scoring system

Groups of organisms	Family	BMWP Score
Stoneflies	Taeniopterygidae Leuctridae, Capniidae, Perlodidae, Perlidae, Chloroperlidae	10
Mayflies	Siphlonuridae, Heptageniidae, Leptophlebiidae, Ephemerellidae, Potamanthidae, Ephemeridae	
True bugs	Aphelocheiridae	
Caddisflies	Phryganeidae, Molannidae, Beraeidae, Odontoceridae, Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae	
Crayfish	Astacidae	8
Odonates	Lestidae, Agriidae, Gomphidae, Cordulegasteridae, Aeshnidae, Corduliidae Libellulidae	
Caddisflies	Psychomyiidae, Ecnomidae, Phylopotamidae	
Stoneflies	Nemouridae	7
Mayflies	Caenidae	
Caddisflies	Rhyacophilidae, Glossosomatidae, Polycentropodidae, Limnephilidae	
Molluscs	Neritidae, Viviparidae, Ancylidae, Acroloxidae, Unionidae	6
Caddis flies	Hydroptilidae	
Amphipods	Corophiidae, Gammaridae, Crangonyctidae	
Odonates	Platycnemididae, Coenagriidae	
Beetles	Haliplidae, Hygrobiidae, Dytiscidae, Noteridae, Gyrinidae, Hydrophilidae, Hydraenidae, Clambidae, Scirtidae, Dryopidae, Elmidae	5
True bugs	Mesoveidae, Hydrometridae, Gerridae, Nepidae, Naucoridae, Notonectidae, Pleidae, Corixidae	
Caddisflies	Hydropsychidae	
Flies	Tipulidae, Simuliidae	
Flatworms	Planariidae, Dogesiidae, Dendrocoelidae	
Mayflies	Baetidae	4
Alderflies	Sialidae	
Leeches	Pisicolidae	
Molluscs	Valvatidae, Hydrobiidae, Bithyniidae, Lymnaeidae, Physidae, Planorbidae, Sphaeriidae	3
Leeches	Glossiphoniidae Hirudinidae, Erpobdellidae	
Isopods	Asellidae	
Flies	Chironomidae	2
Worms	Oligochaeta	1

Source: adapted from Friedrich, Chapman and Beim (1996)

BOX 2.1 PROCEDURE FOR ASSESSING WATER QUALITY USING THE BMWP SCORING SYSTEM IN TABLE 2.3

1. Decide the major habitat types at the sample site to be assessed. For example, weed beds, gravel, and silt substrates all constitute important habitat types to be sampled.
2. Use a standardised collection technique to collect the macroinvertebrates from the selected habitats, e.g., a kick sample (see Box 5.1).
3. Combine the organisms collected from each habitat at a sampling location and treat them as a single sample.
4. Sort the macroinvertebrates in the sample into the families shown in Table 2.3 (e.g., into sensitive families like Ephemeridae, Leuctridae or Heptageniidae which score 10, or highly tolerant families like Oligochaeta which score 1). Even if more than one species is found from a particular family, that family is only scored once.
5. The BMWP score is calculated by adding the scores of all the families found. The BMWP score can then be divided by the total number of families present to give the ASPT.

Example

Baetidae, Sphaeriidae, Assellidae and Oligochaeta were found in a sample. From Table 2.3, the BMWP score is calculated as 4 + 3 + 3 + 1 = 11.

The total number of families in the sample is 4, so the ASPT is calculated by dividing the BMWP score by the number of families: 11 ÷ 4 = 2.75.

The results suggest the water quality is very poor.

Rapid water quality bioassessments in which macroinvertebrates are quickly sorted and identified in the field to obtain an immediate indication of the class of water quality have also been developed and used at national level, e.g., Barbour *et al.* (1999). Some rapid bioassessments are based on the BMWP score and others on a modified national biotic index. They are usually based on the numbers and types of macroinvertebrate species present, and are a cost-effective method of determining water quality.

2.2 Community structure approach

The community structure approach uses the numerical abundance of each species in a community. If one organism dominates an ecosystem, it is usually the most tolerant to the cause of disturbance in the ecosystem. A balanced or healthy ecosystem should have a large diversity (number) of species at each trophic level, with each species represented by few individuals.

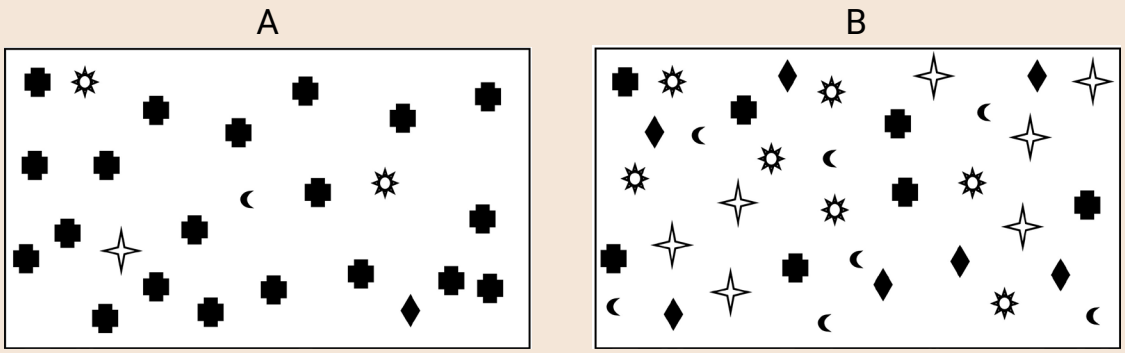
Indices of community structure are broadly applicable to a range of pollutants and geographical areas. They represent the combined effect of all impacts at the site being sampled and, therefore, may not identify any specific cause of the observed changes. An in-depth knowledge of biology and ecology is not needed to interpret the results of community structure indices because the numerical results are mostly used to determine whether the ecological quality of a water body is improving or getting worse, or for comparison between sampling sites. However, a knowledge of taxonomy is still required to identify and count species in the samples.

Changes in the structure of aquatic communities can be monitored with similarity indices by comparing the species present in different sample sites or locations; or with diversity indices that use species diversity and abundance (**Box 2.2**). In both cases, the number of species and their abundance (size of each population) are combined using a mathematical index formula that results in a single numerical value that can be easily interpreted by non-experts (Washington 1984). Some widely accepted examples that are used in freshwater environments are described below.

BOX 2.2 THE CONCEPT OF SPECIES DIVERSITY

Species diversity is a measure of how many different types of taxa are present in communities. It uses both species richness, which is based on the number of species present, and the *evenness* of those species. Evenness is a measure of how homogeneous a community or ecosystem is in terms of the relative abundance of different species. In the hypothetical example shown in **Figure 2.A**, there are two communities (A and B) that both contain five species, hence they have equal species richness. However, community A is dominated by a single species while community B has equal proportions of each species. Evenness is higher when species are present in similar proportions, meaning community B has higher species diversity, because the evenness is higher. The concept of diversity is used as an indicator of ecosystem health and resilience.

Figure 2.A **Two communities with the same five species but different number of individuals of each species. Both communities have equal species richness, but community B has higher evenness than community A**



2.2.1 Similarity indices

Similarity indices compare assemblages of organisms from two different locations or samples. The indices are based on a) the number of species occurring in both samples; and b) the number of species unique to each sample. Although similarity indices are measures of community structure, they cannot give a value for just one site alone and are more suited to determining impacts from point source pollution where unpolluted and polluted sites can be compared. Therefore, the paired sites often comprise an unimpacted control site and a site where an impact on the aquatic community is expected. It is important that the two sites are as similar as possible, i.e., the communities at each site would normally behave and respond in the same way to perturbations. In practice, paired sites are often from the same or similar catchment, or from the same river upstream and downstream of the source of pollution. All habitat features, such as substrate type, flow conditions, level of canopy cover (if any), channel width, etc., should be thoroughly investigated before the final selection of the sites to ensure that the natural physical and chemical characteristics are similar at both sites.

In a similarity index, a value of 1 denotes that the two communities being compared share all their species, while a value of 0 means that they do not share any species. To get the percentage of similarity, the result is multiplied by 100. Similarity indices are not widely used in routine freshwater quality monitoring programmes but may be useful for special surveys and research studies. Detailed descriptions and a discussion of their use in aquatic monitoring are given by Washington (1984). An example is the Jaccard's index, also known as Jaccard's similarity coefficient, which is a simple similarity index. It compares the similarity and diversity of sample sets using presence/absence data and excludes information about abundance (Jaccard 1901).

Jaccard's index $S_j = \frac{(a)}{(a+b+c)}$ (1)

Where a is the number of species found at both sites, b is the number of species found at site B but absent from site A, and c is the number of species found at site A but absent from site B.

2.2.2 Diversity indices

Differences in species diversity within a water body can be used to detect changes in water quality between sites, or over time at the same site. The natural environmental characteristics of a sampling site must be comparable from one sampling occasion to another (e.g., current velocity, substrate structure, temperature, light intensity). Certain types of freshwater habitats can have naturally low diversity due to the physical and chemical constraints of the habitat, such as in headwater streams (Meyer *et al.* 2007). Species diversity can also increase if a disturbance produces more favourable conditions for some species; for example, if nutrient concentrations increase in nutrient-poor waters. For this reason, diversity indices are best used to monitor impacts on water quality when the whole aquatic community is under stress, such as stress resulting from toxic or physical pollution.

There are two underlying assumptions in the use of diversity indices: 1) unpolluted environments have more diverse communities than polluted environments and, 2) there is an increase in the abundance of pollution tolerant taxa in polluted environments. Hence, organic matter pollution should lead to the decline in species richness with a few taxa that are tolerant of low oxygen and high suspended solids becoming abundant. Benthic organisms are best suited for diversity indices in aquatic environments because they are less mobile than fish or plankton, and therefore reflect the quality of the selected location.

Measures of diversity are based on the following assumptions:

- All species are equally important with respect to their ecological role, i.e., there are no keystone species.
- All species are equally detectable.
- Abundance is based on measures that are comparable for all species, i.e., counts of individuals, or biomass of each species, but not a mixture of these measures.

When choosing an appropriate diversity index, the sensitivity of the index to sample size should be considered, as well as the emphasis it places on rare or abundant taxa, and on species richness or species evenness. Some examples are presented below.

The Simpson Index and Simpson's Diversity Index

The Simpson Index *(D)* is one of the simplest approaches to the measurement of diversity because it looks mostly at dominance (Simpson 1949). The Simpson Index is used to analyse biodiversity considering both richness and evenness (see examples in **Box 2.3**). The probability that any two randomly selected species from a sample will be equally likely to occur is given by *D*. As *D* is a probability it ranges from 0 to 1, with 0 representing a very high diversity and 1 representing no diversity. The Index is calculated using the following equation:

$$D = \frac{\sum n(n-1)}{N(N-1)} \quad (2)$$

Where n is the total number of individuals of a particular species, and N is the total number of organisms of all species.

The Simpson's Diversity Index is the reciprocal of the Simpson Index (1 – *D*):

$$= 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right) \quad (3)$$

The value of this index also ranges from 0 to 1 but, in this case, the larger the value, the greater the sample diversity. It is less sensitive to rare species than the Shannon-Wiener Index described below.

Shannon-Wiener Index

The Shannon-Wiener index (H') (Shannon and Weaver 1949) is an information index and is the most commonly used diversity index in ecology. It is similar to the Simpson Index because it takes into account species richness and the proportion of each species within the aquatic community, but it is more sensitive

BOX 2.3 EXAMPLES OF ASSESSING WATER QUALITY WITH THE SIMPSON INDEX (D)

Example 1

The total number of organisms of all species (*N*) and the number of individuals of a particular species (*n*), were counted from a sample and are given below:

Chironomidae larvae *n* = 26; Ephemeroptera *n* = 2; Gammarus *n* = 1

Total *N* = 29

Simpson Index D is calculated using equation (2):

$$D = \frac{(26(26 - 1) + 2(2-1) + 1(1 - 1))}{29(29 - 1)}$$

$$D = \frac{(650 + 2 + 0)}{812}$$

$$D = (652) / 812$$

$$D = 0.80$$

In this example, the probability of choosing two individuals from the sample that belong to the same species is high, indicating that this sample has very low diversity.

Example 2

The total number of organisms of all species (N) and the number of individuals of a particular species (n), were counted from another sample and are given below:

Chironomidae larvae n = 6; Ephemeroptera n = 6; Gammarus n = 7

Total *N* = 19

Using equation (2):

$$D = \frac{6(6 - 1) + 6(6 - 1) + 7(7 - 1)}{19(19 - 1)}$$

$$D = \frac{(30 + 30 + 42)}{342}$$

$$D = (102) / 342$$

$$D = 0.30$$

In this example, the probability of choosing two individuals that belong to the same species is quite low, indicating that this sample has quite high diversity.

to rare species. The index (H') increases as both the richness and the evenness of the community increase.

$$H' = -\sum\left(\left(\frac{n_i}{N}\right) * \ln\left(\frac{n_i}{N}\right)\right) \quad (4)$$

Where n_i is the number of individuals of a species, N is the total number of individuals in the sample, $\left(\frac{n_i}{N}\right)$ is the proportion of the total sample represented by species i, and ln is the natural log.

The calculated Shannon-Wiener Index can then be assigned to one of the following conditions:

- Low diversity: value <1.5
- Medium diversity: value from 1.5 – 2.5
- High diversity: value >2.5

A drawback of the Shannon-Wiener index is that the interpretation of medium diversity (1.5 – 2.5) is subjective. A worked example of the index is given in **Box 2.4**.

Pielou Evenness

Pielou evenness (*J or E*) compares the actual diversity value (such as obtained with the Shannon-Wiener Index) to the maximum possible diversity value (i.e., all species equally common) (Pielou 1966). For the Shannon-Wiener Index *H'*, the Pielou evenness (*J*) is given by:

$$J = \frac{H'}{H_{max}} \quad (5)$$

where H_{max} is ln(S), and S is the total number of species.

BOX 2.4 AN EXAMPLE OF THE SHANNON-WIENER INDEX

The total number of individuals in the sample (*N*) and the number of individuals of a particular species (n_i), were counted from a sample and the results are given below:

Nemouridae n_1 = 10; Chironomidae larvae n_2 = 60; Baetidae n_3 = 25; Heptageniidae n_4 = 1; Asellidae n_5 = 4

Number of species = 5

Number of individuals = (10 + 60 + 25 + 1 + 4) = 100

	n_i /N for each species	ln (n_i /N) for each species
Nemouridae	10/100 = 0.10	ln (0.10) = −2.30
Chironomidae	60/100 = 0.60	ln (0.60) = −0.51
Baetidae	25/100 = 0.25	ln (0.25) = −1.39
Heptageniidae	1/100 = 0.01	ln (0.01) = −4.61
Asellidae	4/100 = 0.04	ln (0.04) = −3.22

Shannon-Weiner Index:

Nemouridae	0.10 x −2.30 = −0.23
Chironomidae	0.60 x −0.51 = −0.31
Baetidae	0.25 x −1.39 = −0.35
Heptageniidae	0.01 x −4.61 = −0.05
Asellidae	0.04 x −3.22 = −0.13

Σ = (−0.23 + −0.31 + −0.35 + −0.05 + −0.13) = −1.07

− Σ = 1.07

H' = 1.07 The sample has low diversity

The value of Pielou evenness is between 0 and 1. Increased variation in abundance between different taxa within the community results in lower values for*J* or *E*. Pilou evenness is limited in its usefulness by its high dependency on sample size and also its high sensitivity to rare taxa. The sampling effort often determines the estimated number of species (*S*) and the sample size, so a good sampling effort is needed to achieve a reliable result for Pielou evenness. A worked example of Pielou evenness is shown in **Box 2.5**.

2.2.3 Multi-metric indices

Multi-metric indices combine different measures of ecosystem condition (Vadas Jr *et al.* 2022). They can require detailed ecological information and are usually region-specific. Therefore, they are not widely used for routine water quality monitoring. An example is the Index of Biological Integrity (IBI) which can be used to evaluate the health of an aquatic ecosystem and water pollution issues. Biological integrity is the ability of an ecosystem to withstand, or to recover quickly from, most kinds of natural and anthropogenic disturbances. Karr (1981) first developed the concept of the IBI to evaluate water quality by examining fish community attributes but other similar indices have been developed for specific organisms, such as algae, macroinvertebrates (Weigel, Henne and Martinez-Rivera 2002) and vascular plants, and for specific environmental conditions or regions, e.g., cold water rivers with low species richness (Mebane, Maret and Hughes 2003) and limestone streams (Botts 2009).

BOX 2.5 AN EXAMPLE OF PIELOU EVENESS

Using the Shannon-Wiener result of H' = 1.07 in Box 2.4

H_{max} is ln(S) where S is the number of species in a sample: *ln* (5) = 1.609

therefore J = 1.07 / 1.609

J = 0.665

The result of 0.665 is closer to 1 than 0 and shows a fairly even community.

An IBI synthesises diverse biological information in order to depict, numerically, associations between human influence and biological attributes (metrics) of an ecosystem. An IBI typically uses between 8 and 12 metrics of a biological assemblage such as taxa richness, community composition (number of species in that community and their relative numbers), trophic structure (those species at similar levels in the trophic structure may share a similar function and food source), reproductive function, tolerance to human disturbance, abundance and condition. Information for these categories is collected at a site and evaluated in comparison with the expected data from an undisturbed (or relatively undisturbed) site located in a similar sized water body, in a similar geographic region. As there are few truly natural sites available anywhere, the natural condition may be estimated from minimally disturbed sites or by using historical data. A numerical value is allocated to each metric based on whether it deviates strongly from, deviates somewhat from, or approximates, expectations from the undisturbed site. The sum of these values then gives an overall site score (Karr *et al.* 1986). A high IBI score indicates that a biological assemblage is like that of a minimally impacted site of comparable size and type, in the same geographic region.

The key steps in developing and using an IBI are:

- Defining what the natural condition in a minimally disturbed area should be.
- Defining biological attributes that change along a gradient of human influence.
- Associating the defined changes with specific human impacts.

An IBI can help to identify potential management practices that could improve the biological integrity, and hence the overall condition of the ecosystem, including its water quality. An example of the application of an IBI using fish in a river is available in An, Park and Shin (2002) and a recent review of use and applicability of multi-metric indices is available in Vadras Jr *et al.* (2022).

Chapter 3

MONITORING CONTAMINANTS WITH BIOTA

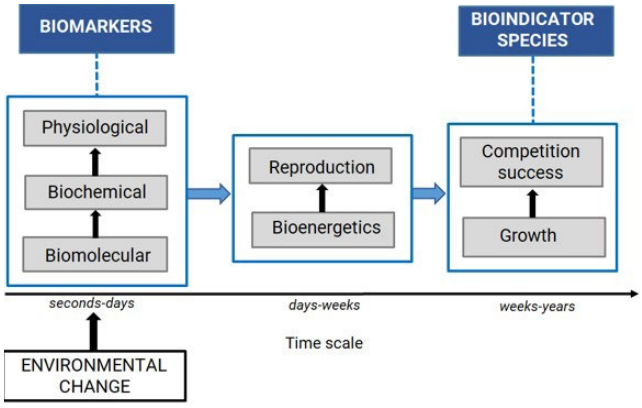
Contaminants introduced to aquatic environments can potentially affect many or all of the organisms present in the freshwater ecosystem. The severity and spatial extent of a pollution incident, long after the pollutant has dissipated, can be investigated by looking at the presence or absence of organisms (as described in chapter 2), or by looking for impacts on individual organisms. However, knowing the impacts of a toxic compound on individual species does not necessarily provide information on the impacts of that compound on the whole ecosystem. In addition, impacts of a pollutant on individuals that occur within days may not result in measurable changes in the whole ecosystem in a water body for months or even years (Boudou and Ribeyre 1997).

The concentration of the pollutant(s), the duration of exposure by aquatic organisms, and other environmental factors, can all influence the severity of the impacts. The main environmental conditions in a water body that can cause stress to an organism, making it more vulnerable to the impact of a toxic pollutant, are shortage of food supply, habitat alterations, sedimentation, drought, or low oxygen concentration. A particular toxin may affect an environmentally-stressed organism at a lower concentration in its native water body, than in laboratory tests.

Tolerance to toxins can be extremely variable between species. Some species may be able to tolerate exposure to a toxin for a short time and appear unaffected, with the impact only becoming apparent some time after the toxin was released into the water body. The length of time an organism is under the influence of a harmful concentration of a substance is known as the duration of exposure. Organisms that are not mobile are subjected to a longer *duration of exposure* than motile organisms, such as fish that may be able to swim away from a source of contamination. The *degree of exposure* to a toxin in a water body depends on its concentration, which may be affected by the mixing pattern following its discharge or release (see accompanying guidebook on "*Water Quality Monitoring and Assessment in Rivers, Lakes and Reservoirs*"). For example, the contaminant may remain on one side of the river in a high concentration close to its discharge and complete mixing and dilution may only occur further downstream.

Many organisms demonstrate some form of stress at sub-organism level when exposed to contaminants or a change in their environment. This stress can result in changes in reproductive ability or poor growth rates, and may not become apparent for some time after exposure (e.g., for weeks, months or longer), depending on the life history of a particular organism (see **Fig. 3.1**). Cellular, biochemical, molecular or physiological changes can be measured in cells, body fluids, tissues or organs using *biomarkers*. Biomarkers indicate exposure of organisms to, and/or effects of, xenobiotic substances (substances not normally found in living tissues). Changes at the molecular or biochemical level can be detected much sooner than changes at the macroscopic level (Fig. 3.1). Examples of biochemical changes at the cellular level are photosynthetic performance, sugar levels in the blood of fish, glycogen in the liver and muscles of fish, and specific enzymes (e.g., cholinesterase). Cellular components such as ATP (adenosine triphosphate)

Figure 3.1 Environmental stress leads to different levels of impact on species over time (after Bellinger and Sigee 2010)



and DNA (deoxyribonucleic acid) can also be useful as indicators of environmental stress in organisms (Jorge *et al.* 2013). Kolarević *et al.* (2016) incorporated the study of genotoxic potential in the multi-national monitoring programme for water quality of the Danube River. The use of biomarkers often requires complex and specialized techniques compared with the use of bioindicator species methods described earlier in Chapter 2 and, therefore, they are mostly used for monitoring for specific impacts rather than routine water quality monitoring.

Organisms that are used for the quantitative determination of contaminants in the environment are known as *biomonitors*. In addition to bioaccumulating contaminants (see section 3.3), they may demonstrate physiological or morphological responses that can be measured as they accumulate contaminants in their tissues. The use of biomonitors for aquatic pollution can help to reduce the time and cost associated with *ad hoc* chemical screening of a water body.

When considering monitoring with biota for toxic pollution, it is important to choose an approach and a method that will enable the monitoring programme to meet its objectives. **Table 3.1** contrasts some of the key features of the use of biomarkers with the use of bioindicators in freshwater quality monitoring. A few examples of some of the more common methods used in water quality monitoring programmes are described in the following sections. It is not possible in this guidebook to describe examples of all the possible approaches and applications of biomarkers and bioindicators.

Table 3.1 Main features of biomarkers and bioindicators in the assessment of environmental change

Major Features	Biomarkers	Bioindicators
Types of response	Subcellular, cellular	Individual – community
Primarily indicates:	Exposure	Effects
Sensitivity to stress	High	Low
Relationship to cause	High	Low
Variability in response	High	Low
Specificity to stress	Moderate-high	Low-moderate
Timescale of response	Short	Long
Ecological relevance	Low	High
Analysis requirement	Immediate, on site	Any time after collection (fixed sample)

Source: adapted from Bellinger and Sigee (2010)

3.1 Principles of toxicity and their use in water quality monitoring

The *toxicity* of a substance in living organisms is dictated by the chemical properties of that substance, i.e., whether it is water or fat soluble, its persistence in the environment, whether it is readily transformed either chemically or biologically to a different compound (which may be more or less toxic), and whether it is readily bioaccumulated in living organisms. Compounds that are readily accumulated in tissues without toxic effects can be useful for biomonitoring of water quality (see section 3.3). There are different mechanisms of toxicity, such as poisoning of the whole organism, damage to particular body tissues or damage to the brain or nervous system, resulting in death, disability or behavioural effects. Some of these effects may not be easily observed or detected without specially designed biochemical or physiological tests.

In aquatic environments, exposure to toxic compounds can occur by several routes, typically through ingestion of contaminated food or by absorption directly from the water, e.g., fish can absorb contaminants as they pass water over their gills. The overall amount of a toxin that an organism is exposed to is known as the dose. The dose depends on factors such as the route of exposure (ingestion, absorption), the length of exposure (seconds, minutes, hours), the duration of the exposure (i.e., does it occur for days, weeks or years), the frequency of exposure (once only, daily), etc. Understanding the route of exposure and the dose that results in adverse impacts on aquatic organisms can assist with establishing Maximum Allowable Toxic Concentrations (MATC) for discharges of substances or indicate the standard to which an effluent needs to be treated prior to discharge. There are national and international databases that make toxicity information available, such as ECOTOX which is made available by the US Environmental Protection Agency (USEPA) (https://cfpub.epa.gov/ecotox/).

Elements and compounds are often described as being of high or low toxicity. Highly toxic compounds are those for which very small amounts cause damage to living tissues. Compounds of low toxicity only result in damage after the concentration builds up to a sufficiently high level in the living tissues. *Acute toxicity* typically occurs when an organism has been exposed to a large dose of a toxin over a short amount of time, causing a rapid negative effect, often resulting in death. This response can be used to determine the lethal concentration of a substance or effluent over a certain amount of time. The concentration that kills 50% of the test organisms within a 48-hour period is described as a 48-hour LC_{50} (lethal concentration (LC) for 50% of test organisms). The results of similar tests that use effects other than death are given as the effective concentration (EC), i.e., 48-hour EC_{50}. Examples of effects in freshwater organisms include immobility or cessation of feeding. *Chronic toxicity* is the exposure of an organism to low doses of a toxin over a long period of time, where the effect can be either lethal, or sub-lethal (i.e., not enough to cause death). For example, many physiological and biochemical effects can be sub-lethal.

Toxicity tests, particularly acute toxicity tests, can provide very fast results, which makes them very effective for monitoring emergencies and accidental pollution events in samples taken from aquatic systems. Although the tests do not confirm the exact chemical that causes the negative reaction in the test biota, they can still provide fast confirmation of the relative toxic impacts and help to focus more quantitative analysis. For example, if a fish-kill occurs, numerous water samples from different locations may be taken for chemical analysis to try to find the cause. However, toxicity tests carried out immediately using water samples from the water body may indicate which sample locations show the greatest toxicity and thus guide the analysts to which samples might provide the most information on the chemicals that could be responsible. This can speed up the process of initiating an appropriate management response.

3.2 Examples of using toxicity in water quality monitoring

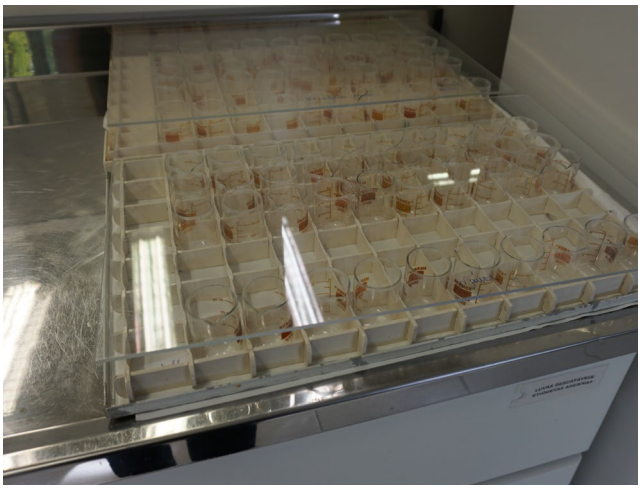
Most toxicological methods are used under controlled conditions in a laboratory, where it is easier to observe the effect on cells or tissues. Tests carried out under laboratory conditions may not reflect the situation in

a natural water body because there are often other environmental factors that influence the response of the organism, such as oxygen concentration and food availability. There are many different types of toxicity tests that are used globally, some of which are standardised for national or international use for known contaminants or water samples taken from water bodies. Examples of tests used to determine toxic effects on specific organisms or on the biotic community, or to determine modes of action (cellular change) and transformation (gene change) are described below.

A *bioassay* is toxicity test carried out under standardised conditions to determine the effect of any substance on any living organism or cell. Bioassays use species that demonstrate a measurable indication of environmental stress caused by substances in the water body. The species used must be capable of living in laboratory conditions, such as microalgae, macroinvertebrates and fish. The rapid growth rates of microalgae, and their susceptibility to dissolved substances that can be absorbed through their cell walls, make them particularly useful as indicators of toxic contamination. A common approach is to grow selected species of freshwater algae in clean water controls and in different dilutions of a contaminated sample from the field or known concentrations of the contaminant. The growth rates of the algae in each treatment are then monitored and the differences recorded. The planktonic crustacean, *Daphnia magna*, is also widely used because it is easy to grow in laboratory conditions and is sensitive to changes in water chemistry (**Fig. 3.2**). These two methods have been standardised by the ISO (ISO 2012a; ISO 2012b).

Bioluminescent bacteria can also be used to detect toxicity. Their respiratory system is affected by the presence of toxic substances, leading to a reduction in their light output. The change in luminescence can be measured, enabling calculation of percentage inhibition, which can then be directly correlated with toxicity. The inhibitory effect of water samples on the light emission of *Allivibrio fischeri* has been standardised by ISO 11348 (ISO 2007a). The measurements can be carried out using freshly prepared, freeze-dried or liquid-dried bacterial preparations. Due to the speed of analysis, and

Figure 3.2 **A laboratory arrangement for bioassays that tests the effects of different dilutions of potentially polluted water on freshwater organisms, such as *Daphnia magna*. © Deborah Chapman**



because full chemical characterisation of the sample does not need to be analysed, this type of bioassay is now used widely for monitoring water quality.

Measuring chlorophyll α fluorescence is an economically feasible, fast, reliable, versatile, non-intrusive technique that indicates the photosynthetic efficiency of aquatic plants and algae, while also providing an approximation of algal biomass. Stress induced by environmental conditions, including the presence of toxins such as herbicides and metals, can alter or completely inhibit chlorophyll α fluorescence. Comparing natural fluorescence with fluorescence produced in the presence of a contaminant can be useful for detecting differences in water quality (Kumar *et al.* 2014). The method gives rapid results, and portable instruments are available enabling fluorescence to be measured in the field, especially in lakes, reservoirs and impounded rivers where phytoplankton populations are abundant. A review of their use is available in Ralf *et al.* (2007).

Long-term toxicity tests can detect the impact of chronic exposure to a toxin in a water body, and can be used to test the carcinogenic, mutagenic and teratogenic capacity of a substance. An example of a standardised test is the reverse mutation assay with

Salmonella typhimurium, or the Ames test (ISO 2012c), which uses bacteria to determine the mutagenic potential of a substance or water sample. Such methods would rarely be used in routine water quality monitoring but may be useful for special surveys, especially in relation to drinking water sources; see for example Lv *et al.* (2015).

3.2.1 Using toxicity for early warning monitoring

The need to protect human health from the presence of contaminants in freshwaters has led to considerable interest in using biota as early warning systems, rather than relying on conventional water quality sampling and chemical testing.

Where there is potential for a water body to contain a range of contaminants at any one time (such as from an accidental spill), a dynamic toxicity test conducted in real time can be useful for alerting water resource managers to potentially harmful changes in water quality. The International Commission for the Protection of the Rhine installed the International Warning and Alarm Plan (WAP Rhine) to protect drinking water intakes on the main rivers and major tributaries (Diehl *et al.* 2005). The monitoring stations use on-line sensors for physical-chemical parameters together with biological sensors using fish, *Daphnia*, mussels, algae or bacteria either alone or in combination (Gerhardt, Janssens de Bisthoven and Penders 2003). The basic principle of early warning biomonitors involves diverting a continuous flow of water from the water body through a specially built apparatus containing the test organisms, with a system to record behavioural changes. For example, changes in water quality can trigger changes in the swimming activity of fish and planktonic crustacea, such as *Daphnia*, and the opening and closing of the valves of mussels (Kramer and Foekema 2001). The movements in response to changes can be detected physically, as in the case of a dynamic fish monitor in which stressed fished can no longer swim against a water flow and are carried backwards against a sensor, or optically where the number of movements detected are recorded. Significant change in recorded movements, e.g., *Daphnia*, can activate an alarm or initiate an action, such as automatic water sampling or closing of a drinking water supply intake.

The potential for the use of single-celled organisms to provide real-time monitoring and assessment for early warning of pollution in water bodies (including the methods mentioned above and the future development of biosensors) has been reviewed recently by Wlodkowic and Karpiński (2021).

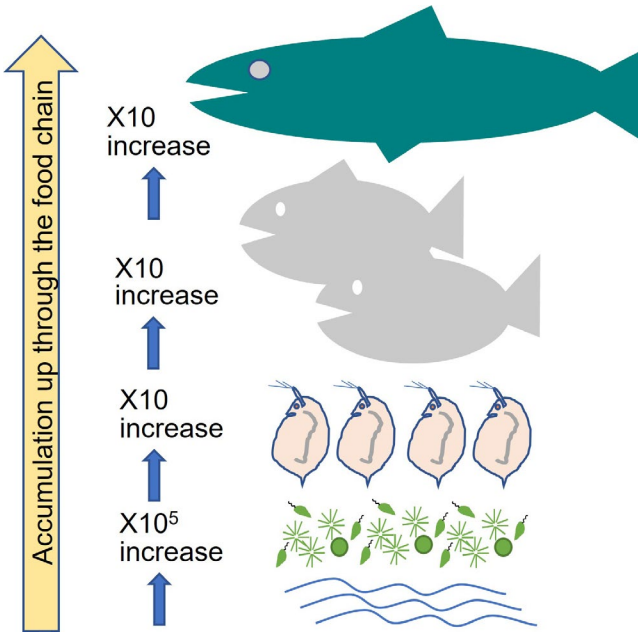
3.3 Monitoring contaminants in biota

The accumulation of contaminants in the tissues of aquatic species is widely used for monitoring the presence and trends in metals and organic micropollutants in water bodies. The extent to which a contaminant is absorbed and potentially *bioaccumulated* by organisms depends on its *bioavailability*. There are many factors that influence whether a contaminant will be absorbed in aquatic organisms. Contaminants that are water soluble may be absorbed directly into living cells, such as microalgal cells, and these cells can accumulate the contaminants until they reach the toxic threshold for the species. Organisms that consume contaminated prey can accumulate high concentrations of some contaminants leading to biomagnification (**Fig. 3.3**). The threshold concentrations for toxicity in the organisms and their tissues can exceed the concentrations in the water in which they live by several orders of magnitude (Fig. 3.3). This can be useful for monitoring the presence of contaminants in a water body when concentrations in water samples are too low for the available analytical techniques. Contaminants that are insoluble in water (principally organic contaminants), but are soluble in lipid, may accumulate in specific tissues of living organisms.

There are many factors that influence the overall accumulation of contaminants, as well as the concentrations in specific body tissues. These are important when selecting an organism and its specific tissue samples for biomonitoring. Examples are:

Diet – For some organisms, their food may provide the main source of contaminants. Diets change according to the availability of the food source (which may be seasonal), and in some organisms with the life stage, because juveniles eat different food from adults. This information is important for selecting appropriate organisms for biomonitoring and for interpreting the results.

Figure 3.3 **Some trace elements and organic compounds in water can accumulate in biota, in which their concentrations are magnified with each step in the food chain**



Age – Older organisms have had a longer period over which to bioaccumulate contaminants and may, therefore, have higher concentrations than juveniles. Moreover, some aquatic organisms can accumulate contaminants into the tissue throughout their lifecycle while others may only accumulate contaminants during some growth stages.

Size – Larger individuals have a greater mass of tissue that may be capable of absorbing greater amounts of contaminants. They can also provide more tissue for the analytical procedure.

Sex – Some organisms store additional fat reserves when preparing for breeding and these reserves may be redistributed to gonads and/or depleted once breeding is over. This could influence the choice of body tissues for contaminant analysis.

Other important factors to consider are:

- Whether to include organisms from one or more trophic levels.
- Whether there is sufficient information available about the diet, life cycle and behaviour of the organisms, such as whether they are migratory or change diet with season.
- Whether to analyze the whole organism or only specific tissues. For small organisms, composite samples of whole organisms may be necessary.
- Whether there are standard methods for extraction and analysis of the contaminants already available.

Organisms for monitoring contaminants can be collected directly from the water body (passive monitoring) or placed in a water body and collected after a period of time (active monitoring). The time of year and number of samples is very important for passive monitoring because it must coincide with the appropriate life cycle stage of the organisms, e.g., avoid times when fish are known to spawn. For active monitoring, live organisms are placed in a specific location (for example in a cage (**Fig. 3.4**)) in the water body and left exposed for a certain period of time to react to any environmental conditions or contaminants present. If taking this approach, test organisms must also be placed in an uncontaminated control site, to detect any potential cage effects. The frequency of such sampling depends very much on the nature of the monitoring and the objectives. The time of year may influence the placement and subsequent removal of test organisms from the chosen sites; for example, very low flow during a dry season would be unsuitable because the organisms may not always be fully submerged in water. It is important to reduce any potential additional stress on organisms when placing them into a test site.

If the concentration of the contaminant in the tissue of organisms correlates well with the concentrations in the water from which they were collected, chemical monitoring of the contaminant in the organisms can be used instead of analysing water or sediment

Figure 3.4 **Small mesh bags that can be used to hold invertebrates in a water body for active biomonitoring of the impacts of contamination. The bags can be weighted to hold them at the bottom. © Deborah Chapman**

samples. Higher concentrations in the tissues of organisms can make analysis considerably easier than lower concentrations in water samples.

All organisms sampled from different locations or water bodies should be of the same species and of comparable age and size to ensure comparability of analytical results. The age, size and sex should be recorded on collection. Ideally, the organisms should be immobile, so that they represent the water quality and contaminants present in the water body or location within a water body from which they were sampled. Dissection may be necessary to obtain specific tissues and organs for analysis. This should be undertaken by trained personnel because it is essential to avoid contamination of the tissues. The dissection area should be sterilized between each sample. Dissection should be carried out within 24 hours of sample collection, otherwise the whole organisms must be frozen. Freezing should be avoided if possible because it presents the risk of the internal organs rupturing. Each sample, replicate, or organ should be labelled according to an agreed laboratory procedure to ensure the samples can be traced back to the original organism. Useful advice for sampling and analysing fish and shellfish for contaminants is available in USEPA (2000). Detailed guidance on the dissection of fish tissues for chemical analysis, including the equipment required, is given in Section C, Chapter 13 of the Department of Environment and Science Monitoring and Sampling Manual (DES 2018).

Small organisms, such as invertebrates, are usually analysed whole, and numerous individuals may be needed to provide an adequate biomass (weight) of material for processing. Typically, 1 g wet weight is required for one composite sample, i.e., usually not less than 15 individuals for larger invertebrates and not less than 30 individuals for smaller invertebrates. Invertebrates are typically processed as three replicate composite samples for each species, with the same number of organisms of similar size in each composite sample (Scudder *et al.* 2008). Collection is normally performed with clean, plastic nets and plastic forceps, with preliminary sorting and cleaning of individuals with deionised water, undertaken in sterile plastic ice-cube trays. Before weighing the sample, excess water should be removed from individuals using lint-free paper wipes (Scudder *et al.* 2008). Results are typically given as $\mu g\ g^{-1}$ dry weight or $mg\ kg^{-1}$ wet weight or dry weight. If results are expressed in terms of wet weight, the whole organism or selected tissue should be weighed. Oven or freeze-dried samples that have been weighed are usually easier to use because they can be ground into a homogenous powder for sub-sampling.

Chemical analysis of biological tissues can be done using standard analytical techniques. For metal analysis, samples are usually digested or dissolved in a concentrated acid (usually nitric acid) in order to release the metals from the tissue into solution. There are several digestion techniques, and the selected method can have a significant effect on the recovery rate of the heavy metals from the biological tissues (Ranasinghe, Weerasinghe and Kaumel 2016). The digest can be analysed using a variety of techniques, such as Atomic Absorption Spectrophotometry (AAS), ICP-MS (Inductively Coupled Plasma Mass Spectrometry), and ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) or ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy).

Organic contaminants are usually associated with fatty tissue and, therefore, lipid percentage or the fat content of the collected organism is often measured during analysis for organic contaminants. Organic solvent extraction is used, followed by procedures to clean the samples and remove lipids and any other co-extracted contaminants. It is possible to analyse a wide range of organic contaminants in biota, including PCBs (polychlorinated biphenyls), pesticides and pharmaceutical compounds, but most methods are time consuming, specialized and expensive, and therefore only included in monitoring programmes with specific objectives for their identification and quantification. In addition, methods are constantly being refined and new methods are being developed, e.g., Cocco *et al.* (2011).

Chapter 4

MICROBIOLOGICAL MONITORING

Microbiological monitoring is performed routinely by many water utilities and government agencies responsible for water treatment and distribution to ensure a safe supply of water for drinking and other domestic and industrial uses (World Health Organization [WHO] 2022). It may also be included in regular monitoring of water bodies used for bathing and swimming, such as in Europe (EC 2006). Discharges and run-off containing domestic sewage and livestock faecal matter can introduce pathogenic species of bacteria, viruses, protozoa and worms that come from the intestines of humans and animals and infect other individuals when they ingest the contaminated water. Women and children are particularly at risk when collecting water from contaminated water bodies for domestic use when they do not have a piped water supply, and when using the water for recreational use, such as swimming, that involves contact with the water. Due to size and physiological differences, women, men and children can be affected differently and the safest approach is to treat the water to remove all microorganisms before domestic use. The organisms of prime concern are the pathogens that reside in, or pass through the intestine, and cause notifiable waterborne diseases, such as the bacteria Verotoxigenic-producing *Escherichia coli* (VTEC) and *Vibrio cholerae* (that is responsible for cholera), protozoans such as *Cryptosporidium* and viruses like hepatitis A. A comprehensive review of the fate and transport of pathogens in freshwater is available in Brookes *et al.* (2004). Natural communities of bacteria, protozoa and fungi also occur in water bodies but they are mostly not pathogenic and are responsible for the self-purification processes that biodegrade organic matter in waterbodies.

This chapter gives a brief introduction to the principles of monitoring micro-organisms in surface and groundwaters for the purposes of determining the risk to human health from using the water directly without treatment, or for determining the extent of contamination of freshwater arising from human and animal faecal matter. Further information and specific methods are available from the ISO and in Standard Methods Committee of the American Public Health Association, American Water Works Association and Water Environment Federation (2023). The range of potential pathogens that could be monitored in freshwater is large and generally not feasible without specialised microbiological facilities. Therefore, the likely presence of faecal pathogens is inferred from the presence of faecal organisms that are excreted in high numbers and are relatively easy to detect and quantify. These organisms are generally not pathogenic themselves and are therefore known as "indicator organisms". Their presence suggests the possibility of other faecal pathogens, but does not necessarily confirm that pathogens are present. Additional confirmatory tests are required to identify and confirm particular pathogens. Coliform bacteria are widely used as indicator organisms but they can be found naturally throughout the environment, as well as in the intestinal tract of warm-blooded organisms such as humans and livestock. Only the coliform, *Escherichia coli*, can confirm the presence of faecal matter because it does not occur naturally anywhere else. For monitoring purposes, the most common microbiological indicators are:

- Total coliform bacteria
- Faecal coliform bacteria (also known as thermo-tolerant bacteria), and

- *Escherichia coli*.

Isolation and detection of some specific pathogens can take several days, weeks, or months, and the methods of isolation and detection are difficult and costly and, even if a certain pathogen is not found in the sample, it does not mean that other pathogens are not present.

4.1 Location and frequency for sampling

As for all monitoring programmes, the sample locations and frequency of sampling are chosen in relation to the objectives of the monitoring programme. Different seasons and different sites in a water body need to be considered carefully because many factors can influence the variability of microbiological species and their densities. Densities may be significantly higher in the summer than in the spring, and much lower in groundwater than surface water. Point sources, such as municipal sewage and industrial discharges, as well as runoff from urban surfaces and agricultural areas, can alter the densities and composition of microbial communities in surface waters. In addition, the concentrations of faecal indicator organisms in rivers can increase during high flows compared with base flows (Kay *et al.* 2005) and during high rainfall events (Tornevi, Bergstedt and Forsberg 2014). If human health is a primary consideration, such as for drinking water sources or bathing waters, it may be necessary to sample daily or weekly with additional sampling following periods of heavy rain and the associated run-off from agricultural land or overflows from sewers.

The principles of selecting sampling locations in surface waters and groundwaters are discussed in the accompanying guidebooks on "*Water Quality Monitoring and Assessment in River, Lakes and Reservoirs*" and "*Water Quality Monitoring and Assessment of Groundwater*". The variability in the distribution of pathogens in water bodies means that the common approach of using one sample location or sampling one side of a waterbody, particularly in rivers (Quilliam *et al.* 2011), may result in classifications of microbial water quality that are not representative of the location. The efficiency of the sampling regime, particularly for microbiological parameters like faecal indicator bacteria, can be improved by sampling at stations across the width of the river channel, particularly in large rivers, as discussed by Chapman *et al.* (2016).

4.2 Sampling and logistics

Procedures for quality assurance of sampling and analysis for microorganisms are discussed in the accompanying guidebook on "*Quality Assurance for Freshwater Quality Monitoring*". Some important precautions are highlighted in **Box 4.1**.

BOX 4.1 PRECAUTIONS FOR SAMPLING WATER BODIES FOR MICROBIOLOGICAL ANALYSIS

ALWAYS:

- ✓ Collect microbiological samples before collecting other samples.
- ✓ Label the sample bottle before sampling.
- ✓ Use a clean, sterile sample bottle.
- ✓ Discard damaged or contaminated sample bottles. If in doubt, put them aside and take samples in new bottles.
- ✓ Use clean sterile gloves at each location.
- ✓ Wash hands thoroughly before and after collecting samples.

DO NOT:

- × Contaminate the sample bottle by touching the inside.
- × Contaminate the sample bottle lid by touching the inside rim.
- × Rinse the sample bottle before filling it.
- × Put the sample bottle lid on the ground while sampling.
- × Transport drinking water samples with other water sample types e.g., sewage samples.

It is particularly important when sampling for microbiological analysis that the sample is taken from below the surface because, in many still waters such as lakes and reservoirs, the concentration of microbes in the surface film can be 1,000 times higher than below the surface. In addition, the subsurface sample should not be contaminated with any surface film as it is removed from the waterbody. The sample bottle may be attached to a sampling pole to avoid contamination from the surrounding water and introduced to the water upside down to the predetermined location and water depth, and then turned upwards to allow it to fill. If there is a current in the water body, the bottle should be held facing upstream to fill it. If samples must be taken in shallow water, great care must be taken not to disturb the sediments (ISO 2006a).

Most microbiological analyses take at least 24 hours and therefore adequate time must be allowed between sampling and delivery to the laboratory. Ideally, samples should be delivered as soon as possible, or within 6 hours of commencing sampling. Under exceptional circumstances, the sampling and transport time may exceed 6 hours but should never exceed 24 hours. Following collection, water samples should be transferred immediately to a chilled, insulated container preferably at a temperature between 1 °C and 4 °C, unless a particular procedure specifies different conditions (such as freezing for some viruses). Freezer ice packs or loose ice can be used to chill the sample container. The low temperature is used to prevent the multiplication of bacteria during transport which may result in false bacterial counts. Cool and dark conditions should be maintained throughout transport to the laboratory. Microbiological samples should always be protected from sunlight (ISO 2006a).

All the consumables (e.g., laboratory agents) and equipment required must be gathered and prepared prior to sampling. All sampling and laboratory equipment (e.g., sample bottles, pipette tips, spreaders, loops, filters) must be sterilised to avoid contamination of the sample. Re-usable sampling bottles can be autoclaved or sterilised with gamma rays or with ethylene oxide. Sterilised bottles can be kept in a protective bag and only opened immediately before sampling. No contact should be made with the interior or rim of the sample bottle, the cap, or any other surfaces at the sample site (e.g., the ground or work bench). The sample bottle must also be of a sufficient volume for the subsequent analyses that will be carried out. For some analyses, the volume of sample required is very high, e.g., viruses, *Salmonella* sp., amoebae, *Cryptosporidium* oocysts and *Giardia* cysts.

4.3 Enumeration techniques for bacteria

There are many different techniques for the detection and enumeration of microbiological contamination in water samples, many of which have been standardised by ISO, such as those for coliform bacteria (ISO 2012d, 2014) and protozoans (ISO 2006b). The most commonly used techniques for coliform bacteria and *E. coli* are membrane filtration followed by incubation and colony counts, and enzyme substrate tests in which chromogenic and fluorogenic substrates detect enzymes produced by the bacteria (Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation 2023). Enumeration in enzyme substrate tests is based on the most probable number (MPN) technique (ISO 2012d). The advantages and disadvantages of these two techniques are given in **Table 4.1**.

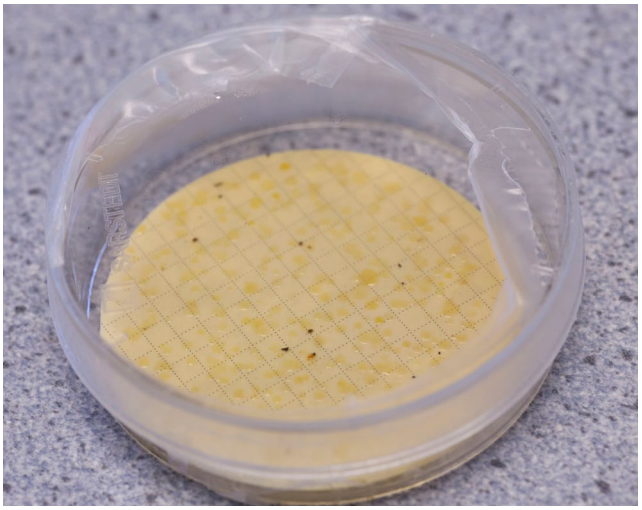
In the membrane filtration method, a minimum volume of 10 ml of the water sample (or dilution of the sample) is introduced aseptically into a sterile or appropriately disinfected filtration assembly containing a sterile membrane filter (nominal pore size 0.2 µm or 0.45 µm). A vacuum is then applied, and the sample is drawn through the membrane filter (**Fig. 4.1**). All indicator organisms are retained on or within the membrane filter, which is then transferred to a suitable selective culture medium in a Petri dish. The Petri dish is then incubated at the required temperature and time (typically 18–24 hours) to allow the growth of the indicator organisms. Visually identifiable colonies in the Petri dish are then counted, and the results expressed in numbers of "Colony Forming Units"

Table 4.1 **Advantages and disadvantages of membrane filtration versus enzyme/chromogenic substrate methods for the enumeration of bacteria in water samples.**

Method	Advantages	Disadvantages
Membrane Filtration	Relatively cheap. Sample volumes greater than 100 ml can be tested. Quite accurate.	Labour intensive. Does not work well for high turbidity samples. Requires training.
Enzyme/Chromogenic Substrate	Can tolerate high turbidity samples. Easy and quick. Unit-dosed packaging eliminates media preparation.	Can be expensive. The level of accuracy may not be adequate in all circumstances. Limited to 100 ml samples.

Figure 4.1 **A simple filtration apparatus that can be used for filtering bacteria from measured water samples. © Patrick Cross**

Figure 4.2 **Colonies of faecal bacteria growing on a membrane filter with a nutrient medium. Each colony appears as a yellow patch. © Patrick Cross**



(CFUs) per 100 ml of original sample (**Fig. 4.2**). Full details are available in ISO (2014). The volume of sample required for membrane filtration is dependent on the anticipated level of bacterial contamination and the type of analysis to be performed. For example, cleaner waters such as drinking water, require a larger sample volume as indicated in **Table 4.2**, and waters with high concentrations of bacteria, such as water contaminated with sewage, may require dilution to obtain a minimum 10 ml sample volume.

The enzyme substrate or chromogenic substrate method is based on enzyme detection and can produce either quantitative or presence/absence results. The method is based on the principle that total coliforms have the enzyme β-D-galactosidase which hydrolyses ortho-nitrophenyl-β-D-galactopyranoside (ONPG) and turns yellow and *E. coli* has the enzyme

Table 4.2 Examples of sample volumes required for membrane filtration for faecal coliform analysis of different water sources

	100 ml	50 ml	10 ml	1 ml	0.1 ml	0.01 ml	0.001 ml
Drinking water	X						
Wells and springs	X	X					
Lakes and reservoirs	X	X					
Water supply intake		X	X	X			
Bathing areas		X	X	X			
River water				X	X	X	
Farmyard run-off				X	X	X	
Raw sewage					X	X	X

Source: based on Forster and Pinedo (2016)

β-glucuronidase which hydrolyses the fluorescent substrate 4-methylumbelliferyl-β-glucuronide (MUG). This method is mostly undertaken using commercial kits where the enzyme substrate is mixed with 100 ml incubated at 35 °C for a period of 24 hours. The kits include trays with multiple small wells that can be sealed and placed in the incubator (**Fig. 4.3**) to enable quantification using the most probable number (MPN) method. The number of total coliforms is determined from the MPN table by the number of coloured wells equal to, or in excess of, the comparator sample provided. The number of *E. coli* per 100 ml can be estimated after viewing the tray under an ultraviolet light lamp and counting the number of fluorescent wells equal to or greater than the comparator sample.

Figure 4.3 Multiple-well trays in an incubator. Each tray contains a 100 ml sample mixed with an enzyme substrate to enumerate total coliforms and *E. coli*. © Environmental Research Institute (ERI), UCC

Chapter 5

SAMPLING BIOTA

This chapter introduces some of the common techniques for sampling biota and that may be used in the monitoring approaches described in the previous chapters and for collecting biota as part of a preliminary survey (for an explanation of the need for preliminary surveys see "*Introduction to Freshwater Quality Monitoring and Assessment*"). Biological communities and individuals within a water body often have a non-uniform or clustered distribution. A preliminary survey may be required to determine this and then to assist with decisions on the number of samples needed for a specific habitat within a water body to meet a certain degree of precision and confidence (Friedrich, Chapman and Beim 1996). Understanding the life history, natural range, location and spatial distribution of the species or communities used for monitoring assists with selecting the most appropriate sampling and data analysis methods. Many approaches have specific methods for collecting samples, and it is important to adhere to those methods to ensure reliable results that are comparable between locations and with other monitoring programmes. There are several text books devoted to the topic of sampling and data analysis for ecological survey methods, such as Fowler, Cohen and Jarvis (1998) and Manly and Navarro Alberto (2015).

Biota can be sampled using quantitative, semi-quantitative and qualitative methods. Qualitative methods usually give an indication of species presence or absence and relative abundance from one sampling occasion to another. For example, presence may be recorded as rare, common, abundant, etc., without specific counts. Semi-quantitative methods produce results such as "less than 5", "between 5 and 20" or "greater than 20" individuals, while quantitative methods produce an actual number of a particular species per unit area.

Different groups of organisms from different aquatic habitats may be required, depending on the monitoring approach (see previous chapters). Each group has specific methods for sample collection. The more common methods are described in more detail in sections 5.2 to 5.5 and are summarised in **Table 5.1**, together with their associated advantages and disadvantages that need to be considered before final selection of the method for use in a monitoring programme. Wherever possible, a standardised method should be used.

5.1 Sample site selection and sampling frequency

Sampling frequency and location depend largely on the objectives of the monitoring programme and any specific requirements of the chosen biological technique. For example, the frequency of sampling following an accidental discharge of contaminants may be high (e.g., daily sampling). This high frequency sampling following an accident may only last for several weeks depending on the seriousness of the incident and the duration of the effects. The sampling frequency for early warning systems that use biomonitoring techniques, such as fish biomonitors, must be very high and is often continuous, i.e., by diverting water from the water body of interest through the test apparatus (see chapter 3).

Table 5.1 Comparison of sampling methods for aquatic biota

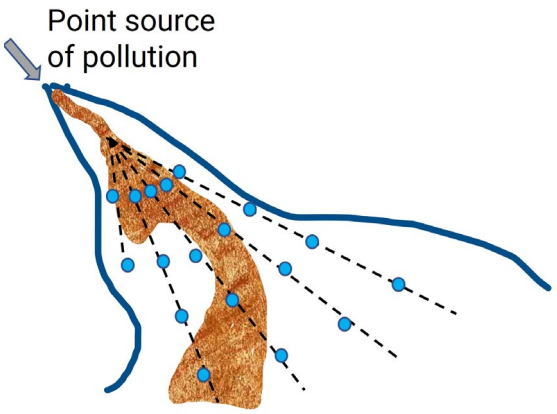
Sampling method	Most suitable organisms	Most suitable aquatic habitats	Advantages	Disadvantages
Hand net	Benthic invertebrates.	Shallow river beds or lake shores.	Cheap, simple.	Semi-quantitative. Mobile organisms may avoid net.
Plankton net	Phytoplankton (60 µm mesh), zooplankton (150 – 300 µm mesh).	Open waters (lakes, reservoirs and ponds).	Cheap and simple. High density of organisms per sample. Large volume of integrated samples possible.	Semi-quantitative Quantitative if fitted with a flow meter.
Bottle sampler	Phytoplankton, zooplankton, microorganisms.	Open waters.	Quantitative. Enables samples to be collected from discrete depths. No damage to organisms.	Expensive unless manufactured in house. Low density of organisms per sample. Small total volume sampled.
Benthic grab sampler	Benthic invertebrates.	Sandy or silty sediments, weed zones.	Quantitative sample. Minimum disturbance to sample.	Expensive. Usually requires winch for lowering and raising.
Corer	Microorganisms and benthic invertebrates.	Sediments, usually in lakes.	Discrete, quantitative samples possible with commercial corers.	Expensive unless made in house. Small quantity of sample.
Artificial substrates	Periphyton, attached invertebrate species, benthic invertebrates.	Open waters of rivers and lakes, weed zones, bottom substrates.	Semi-quantitative compared with other methods for similar groups of organisms. Minimum disturbance. Cheap.	"Unnatural habitat" therefore may not be truly representative of natural communities. Positioning in water body important for successful use.
Poison e.g., rotenone	Fish	Small ponds or river stretches.	Total collection of fish species in area sampled	Destructive technique.
Fish net/trap	Fish	Open waters, river stretches, lakes.	Cheap. Non-destructive.	Selective. Qualitative unless mark recapture techniques are used.
Electrofishing	Fish	Rivers and lake shores	Semi-quantitative. Non-destructive.	Selective technique according to electric current used and fish size. Expensive. Safety risk for operators.

Source: adapted from Friedrich, Chapman and Beim (1996)

Ecological monitoring is commonly used as a means of long-term trend assessment of biological water quality by carrying out annual sampling campaigns in set locations and at the same time of the year. To determine whether there is any seasonal variation in the organisms found at a particular sampling location, a baseline or preliminary survey can be carried out with monthly or seasonal sampling over a full year. Ideally, at least 20 samples are needed for statistical analysis. The frequency of sampling, especially when carrying out an initial baseline survey, must consider the life cycle of the organisms being sampled and the time it may take for their population to recover after the disturbance resulting from sampling. Regardless of the objectives of a monitoring programme, the methods of data collection must be appropriate and consistent within the monitoring programme to allow meaningful comparison between water bodies or within the same water body; see for example Carter and Resh (2001).

For some investigations a control site is needed to make a comparison with sites where impacts on biota are anticipated. A control site should be where the organisms are not exposed to stress caused by human activity. In practice, a control site may be in a different part of the water body or catchment less affected by human activities but expected to have similar species and communities. Where a suspected pollution gradient may exist (and hence different degrees of impact on the biota), sites should be at intervals downstream of a discharge point in a river or along a grid radiating from the discharge point (**Fig. 5.1**).

Figure 5.1 **Sampling to establish the dispersion and dilution of a point source of pollution. Samples are taken at points along an array of transects**



Additional information, such as river flow rate, physicochemical data, and information about the climate and geology of a water body, is often collected as part of a biological monitoring programme to aid interpretation of the biological data. Ideally, biota samples should be collected from the same locations as where hydrological, chemical, and physical monitoring are carried out. Comprehensive field notes that describe the habitat and substrate, as well as the hydrological and climatological conditions at the time of sampling, are critical (**Fig. 5.2**). Any change to the characteristics of a site that is repeatedly sampled, or noticeable differences between sites that are to be compared, could affect the interpretation of the biological data obtained.

Figure 5.2 **Example of a field record sheet that provides useful information for interpreting the results of sampling biota during a biological monitoring programme. © Deborah Chapman**

Field Record Sheet

Name of river/lake ..... **Site code/co-ordinates**.....
Date ..... Time .....

WEATHER CONDITIONS
Air temp (ºC) .....
Rain None ... Showers ... Persistent ... Duration .....h/24 h
Wind Strong ... Moderate ... Light ... Calm/no wind ...
Cloud cover 100% ... Mostly cloudy ... Mostly clear sky ... No cloud ...

VISUAL ASSESSMENT
Catchment Mountainous ... Moorland ... Agricultural ... Urbanised ...

Inputs Rivers ... Number .... Streams ... Number .....
Farm drainage ... Wastewater outlets ...
Other ... Description .....

Shoreline/margins Rocky ... % of shoreline .....
Weed and/or reed beds ... % of shoreline .....
Trees and shrubs ... % of shoreline .....
Artificially constructed banks ... % of shoreline .....

Substrate
Stony ... with algal cover ... without algal cover ...
Silty ... Organic detritus ... Filamentous algae ... Floating macrophytes ...
Rooted macrophytes ... Submerged ... Emergent ...

Water quality
Turbidity None ... Moderate ... Strong ...
Colour None ... Brown ... Green ...

Additional comments .....
.....

PHYSICO-CHEMICAL MEASUREMENTS (at approx. 30cm depth)
Temperature (ºC) ..... Dissolved oxygen (mg l^{-1}) ..... (% sat^{n}) .....
Conductivity (µS cm^{-1}) ..... pH .....
Secchi depth (m) .....

Comments .....

***IN-SITU* CHEMICAL ANALYSIS OR SAMPLE BOTTLE CODE**
Description of sample collection site.....
.....
Phosphorus: Depth taken (m) ..... Measurement/Sample ID..... Units .....

Nitrogen: Depth taken (m) ..... Measurement/Sample ID..... Units .....

ADDITIONAL COMMENTS .....
.....

The suitability of potential sample sites may also need to be checked before the monitoring programme commences, particularly with respect to accessibility and safety. Health and safety measures should be incorporated at the design phase of the monitoring programme. Collecting biological samples from aquatic habitats can be dangerous, and field personnel can encounter a wide range of hazards while in the field. These hazards may differ for men and women and it is important to understand these differences when encouraging female participation in field monitoring. It is therefore important that field personnel are appropriately trained to recognise and deal with hazards as they are encountered. Minimum training should include water safety and first aid, and field technicians should carry first aid kits with them at all times. Depending on national Health and Safety legislation in the country where sampling is to take place, a Risk Assessment may also be required before commencing any field sampling. Further details are available in the accompanying guidebook on "*Quality Assurance for Freshwater Quality Monitoring*".

5.2 Benthic macroinvertebrates

Benthic macroinvertebrates inhabit the sediment and other substrates at the bottom of rivers, lakes, reservoirs and wetland ecosystems. Samples can be collected actively by:

- Disturbing the habitat and collecting the disturbed invertebrates that are flushed out.
- Removing whole sections of the habitat using grabs and cores.
- Placing artificial substrates in the environment and leaving them for a period of time to allow colonization by invertebrates.

Depending on the method used and the objectives of the monitoring programme, analysis can either be performed at the collection site, or the samples can be preserved and transported to a laboratory where a more thorough and accurate identification can be undertaken.

A kick sample with the aid of a hand net is a common and simple method of collecting a semi-quantitative sample of stream invertebrates. A standard length of time should be used when kicking the substrate (**Box 5.1**). A quantitative sample can be obtained with a Surber sampler (**Fig. 5.3**). It can be used in streams and rivers of up to 30 cm depth and with flow rates greater than 10 m s^{-1}. At lower stream velocities and greater depths, some organisms may be washed around the side of the sampler and not be included in the sample. The sample area is limited to the base size of the Surber frame, which is usually 30 cm^2.

The Surber sampler is placed on the stream substrate with the 600 µm mesh net opening facing upstream. Organisms are sampled by first cleaning/rubbing any large stones to remove any attached or clinging invertebrates so that they flow into the net downstream. These large stones are then discarded from the sampling area. The substrate within the Surber frame is then disturbed, usually with a hand trowel, for a set time (typically 1 minute) to dislodge the remaining invertebrates within the sample area.

Figure 5.3 An example of a Surber sampler. © Patrick Cross

BOX 5.1 BASIC PROCEDURE FOR CARRYING OUT A KICK SAMPLE FOR BENTHIC INVERTEBRATES IN A STREAM

1. The hand or pond net is held vertically with the straight edge against the substrate and with the opening facing upstream (**Fig. 5A**).

2. The substrate and stones directly upstream of the net are disturbed and dislodged using the heel and toe of the sampler's boot so the invertebrates are washed into the net (Fig. 5A). This is performed for a set time depending on the sampling objective; sampling riffles would normally require a 1-minute kick sample, while multi-habitat (riffles, weeds, pools etc.) sampling would typically require a 3-minute kick sample.

3. The sample must be transferred from the net into either a clean white tray for immediate identification (**Fig. 5B**), or a container for transport to a laboratory. For transport, the sample should be transferred into a labelled container or bag, making sure that the vessel is clean and large enough for the whole sample, and with a lid (if using a container) that can seal to prevent loss of organisms or liquid during transport. The sample should be preserved (see section 5.2.1) before transportation and storage at the laboratory. This must be done with care to ensure all the organisms are transferred to the container, and they are transported with as little physical damage as possible, because physical damage can make identification difficult.

4. Woody debris and large stones should be discarded once they have been checked and scraped clean of organisms attached or clinging to them.

5. The inside of the net should be carefully checked, and any organisms found should be gently removed and placed in the tray or container.

6. The net should be thoroughly rinsed before the next sample is taken to prevent transferring organisms from one site to another.

Figure 5A Taking a kick sample in a shallow stream. © Deborah Chapman

Figure 5B The contents of the net are emptied into a white tray for sorting, identification and counting. © Deborah Chapman

Care should be taken to ensure that organisms do not escape under the frame. The sample is collected in a bottle attached at the tail of the sampler (Fig. 5.3), which is easily removed for field or laboratory analysis. As with the kick sample, the inside of the net should be carefully checked, and any organisms found should be gently removed and placed in the sample bottle.

Grab samplers are typically used in lakes, reservoirs, or large rivers to collect benthic macroinvertebrates. They are lowered, either manually or using a winch, to the lake or riverbed. Additional weight may be incorporated in the design to increase the speed of descent and the degree of penetration into the substrate. At the bottom, the jaws of the grab are closed to take the sample, and the grab is hauled or winched to the surface (**Fig. 5.4**). Once retrieved, the sample is deposited into a tray or bag for processing and/or transport to a laboratory. Like the Surber sampler, grab samplers are quantitative sampling methods, with a standardised area of substrate.

There are different types of grab samplers for different types of substrates, ranging from mud and silt to coarse sand and gravel. A disadvantage of benthic grab samplers is that they can be expensive to purchase, and some are very heavy and require a winch mounting on a boat for their deployment.

Corers provide quantitative samples of fauna living in soft sediments at depth, with minimum disturbance of the sediment column. This can be useful if depth profiles in the sediment core are required. Corers can be hand-driven into the sediment and heavy weights can be attached to assist in providing the driving force and increased penetration into the sediment (**Fig. 5.5**). Some corers have a weight (messenger) that can be sent down the line to trigger a spring-mechanism to close the corer trapping a column of sediment and the water above it (**Fig. 5.5**).

5.2.1 Preserving, transporting and storing invertebrates samples

Identification of aquatic organisms is almost always much easier when they are still alive because movement, body appendages, colour, etc., can aid identification. Therefore, analysis of invertebrate samples should be undertaken immediately after collection but, if this is not possible, the samples can be transported in chilled cool boxes (2–5 °C), or preservatives such as 70–80 per cent ethanol can be

Figure 5.4 A Van Veen benthic grab sampler with jaws open (left) and closed (right). © Patrick Cross

Figure 5.5 Removing an intact sediment core from Lake Nganoke, New Zealand. By Susie Wood Lakes380, Licenced by CC BY-SA 4.0, via Wikimedia Commons

added. It is important to consider the volume of water and debris in the sample when adding the ethanol to ensure proper preservation. It is useful to take a strong solution of ethanol (e.g., 95 per cent) into the field and to add enough to ensure a final strength in the sample of about 70 per cent. There should also be an equal amount of ethanol to the volume of sample material. Preservatives can cause some invertebrates to constrict, which hinders identification. This can be avoided by adding a relaxant to reduce constriction. It is important to remove as much debris (e.g., stones, wood) as possible from the sample before transportation, because the movement of organisms amongst the debris in transit can lead to damage to their bodies, such as squashing or body appendages being broken off, making identification more difficult in the laboratory. Guidance on preservation and handling of water samples, including samples of biological material is available in ISO (2018).

Some organisms, such as annelids, do not preserve well using ethanol, and it may be necessary to fix them using a 5–10 per cent formalin solution. There are serious health and safety considerations when using formalin because it is a known human carcinogen, and should be avoided whenever possible. If use of formalin is essential, every effort should be made to minimise the risk of exposure for the user.

5.3 Plankton

Plankton can be sampled using a variety of bottle samplers and nets. The choice of sampler depends on the objectives of the monitoring programme and whether a quantitative or semi-quantitative sample is required. If enumeration of species is required, a quantitative sampling method is necessary to express the results as numbers of organisms per unit volume (e.g., number per litre). Qualitative samples can be used when the objective is to obtain a relative abundance of all species present. Determining the species and their relative abundance is often important if the water is going to be used for drinking or for recreation, because some species can produce toxins and others can be difficult to remove during some types of water treatment.

Quantitative plankton samples can be collected using jugs or sample bottles of known volume at prescribed depths in a similar way to the collection of water samples (ISO 2023). Sample bottles need to be weighted at the bottom to ensure they stay vertical in the water column. Grab samplers vary in size and usually comprise a vertical container with openings at each end that can be closed, although some comprise horizontal tubes for sampling shallow waters. The closure mechanism is held open while the sampler is lowered to the required depth on a rope or wire that is marked at metre intervals (note that the length of the rope should be calibrated regularly to ensure that it has not stretched over time). Closing can be activated remotely by sending a weighted messenger down the rope or wire, or by pulling on a separate line. The closure mechanism seals the bottle at the

required depth before it is brought to the surface (**Fig. 5.6**). Plankton samples can also be taken between prescribed depths in the water column by using a simple tube or "hosepipe sampler" (DES 2018).

Nets can be used to collect quantitative, semi-quantitative or qualitative plankton samples. The size of the net mesh depends on the plankton groups of interest. For example, phytoplankton can be sampled with a 60 µm mesh, while different zooplankton groups need mesh sizes between 150 µm and 300 µm. Known volumes of water collected with a bottle sampler, or a pump, can be passed through a filter cup with a pre-selected mesh size to obtain quantitative results (**Fig. 5.7**). The plankton trapped on the filter are backwashed into a sample bottle for transport to the laboratory for analysis.

Figure 5.6 **A bottle sampler that can be lowered to a required depth before being triggered to close by a heavy messenger that travels down the suspension cable. The bottle is brought to the surface to be emptied. © Deborah Chapman**

Figure 5.7 **Obtaining a quantitative plankton sample by filtering a measured volume of water through a filter cup with a plankton mesh of known pore size. © Deborah Chapman**

Larger volume samples, such as when plankton densities are low, can be collected with a specially constructed conical plankton net of the desired mesh size (**Fig. 5.8**). To collect a quantitative sample a flow meter is mounted at the opening of the net to estimate the water volume that passes through the net while the net is being towed through the water. The net can be towed from a boat or lowered and raised through the water column from a fixed structure such as a pontoon. Nets can be raised vertically or towed obliquely to collect a composite sample through the water column or towed horizontally to collect a large volume sample at a single depth. A semi-quantitative sample can be collected by measuring the area of the net opening and multiplying it by the distance that the net is moved through the water to collect the plankton. The plankton from the sides of the net are rinsed into the collection bottle at the end when the net is removed from the water. The fine mesh of the plankton net can easily become clogged resulting in a reduced filtration efficiency. If this occurs, a fresh sample may need to be taken with a reduced towing time.

Quantitative zooplankton samples can also be collected using specially designed traps of known volume, such as the Schindler-Patalas trap (**Fig. 5.9**). These traps are used to sample shallow water or

Figure 5.8 Collecting a qualitative plankton sample by drawing a plankton net through the water column of a reservoir. © Patrick Cross

surface water, they operate in a similar way to bottle samplers and can also be triggered to close at a selected depth.

5.4 Fish

Fish are often used in biological monitoring as bioindicator species, as a component of ecological communities, as biomonitors, and in controlled biotests (see Chapter 3). Before undertaking sampling, it is very important to understand any local or regional regulations relating to removal of fish from water bodies. Restrictions on their capture and treatment may apply and permits or licences may be required. A thorough understanding of the ecology of the target species, and the environmental constraints on

Figure 5.9 Collecting a quantitative zooplankton sample with a Schindler-Patalas trap (Department of Environment and Science 2018) By State of Queensland, Licenced under CC BY 3.0 AU

fish abundance and diversity is also required when deciding whether to include fish in a water quality monitoring programme.

Common approaches to sampling fish include using hooks, nets (**Fig. 5.10**), traps, ichthyocides (poisons specific to fishes) and electro-fishing (**Fig. 5.11**). The way in which the fish will be used for biological monitoring will govern the most appropriate sampling technique, particularly whether fish are required alive for weighing and measuring, or dead for examination of histological impacts or analysis of contaminants in body tissues (see section 3.3). If the reason for collecting fish samples is to analyse their tissue for contaminants following a fish-kill, then samples of sick or dying fish are preferable to dead fish, because the tissue will still be fresh and unaffected by decomposition processes. If this is not possible, the fish which are least decomposed should be collected. Organisms should be put in resealable bags with clear, informative labels on them and stored in a cool box until they are brought to the laboratory for dissection. Further information is available in ISO (2007b).

Non-destructive, quantitative estimates of fish populations can be undertaken using electro-fishing in discrete sections of a water body isolated with stop nets (European Committee for Standardization [CEN] 2003). For rivers, stop nets are placed across

Figure 5.10 Using a gill net to sample fish in a reservoir. © Deborah Chapman

the width at the upstream and downstream points of the section of river being sampled. Starting from the downstream stop net, the fishing team start moving upstream slowly, moving the electrode sideways from bank to bank to the upstream stop net (**Fig. 5.11**). The electric current passing through the water temporarily stuns the fish, allowing them to be picked up easily in hand nets and placed in buckets for immediate weighing, measuring, etc. before transfer to aerated holding tanks. The same area is normally sampled three times. The stop nets should be checked between each sample run, and any fish in the nets collected for processing. To avoid unnecessary stress for the fish, they must be returned to the same area where they were caught as soon as possible after all fish have been removed and recorded.

Seine nets are commonly used in wadable water bodies but can also be deployed from boats in deeper water. The method provides a semi-quantitative assessment of numbers of fish present in the area enclosed by the net. It is often difficult to ensure all fish within the net are captured and therefore the errors associated with the estimated fish population can be quite large. Seine nets have floats attached at the top and weights at the bottom to keep the net vertical in the water. The net dimensions depend on the depth and size of the study area and the mesh size is determined by the target species. The net is deployed by forming a "u-shape" in the water and slowly closing it against the shore. The captured fish are removed to an aerated tank for processing as required by the monitoring programme objectives.

Figure 5.11 Electro-fishing is a non-destructive method for obtaining a quantitative estimate of fish populations. © Deborah Chapman

5.5 Macrophytes

Macrophytes are plants that are rooted in, or attached to, the substrate of a water body. Different species of macrophytes may be fully submerged, have parts that emerge from the water or have surface-floating components (see guidebook on "*Water Quality Monitoring and Assessment in Rivers, Lakes and Reservoirs*"). Quantitative macrophyte sampling methods typically include line intercepts or quadrats (Titus 1993; Madsen 1999). The line intercept method records the linear distance that a species occupies along a line attached between two known points. Quadrats are square, sometimes rectangular, in shape and are positioned on the water body substrate. The positioning of quadrats is either chosen at random or at a predetermined point along a transect line, depending on the monitoring programme design. The percentage cover of each macrophyte species within the quadrat can be estimated. Sampling protocols are described by Parsons (2001) and a review of approaches to monitoring and assessment methods with macrophytes is available by Madsen and Wersal (2017).

5.6 Periphyton

Periphyton include organisms (e.g., algae, filamentous bacteria, zoogloœa bacteria, rotifers and protozoa) that grow on submerged surfaces, such as rocks, sticks and submerged macrophytes. Diatoms, in particular, can be beneficial for water quality assessment because they are immobile or have very limited mobility, and their response to pollution can be very rapid (Masouras *et al.* 2021). Most periphyton have distinct seasonal cycles, so this must be considered during the sampling design process. They may also be subject to scouring during high flow conditions in streams and rivers. Quantitative periphyton samples are usually collected from natural substrates (e.g., stones), but artificial substrates are also often used. The periphyton can be gently removed from the known area of the surface of substrate with a small soft brush, such as a toothbrush, into a collecting container (**Fig. 5.12)**. A common artificial substrate approach is to use conventional glass microscope slides arranged vertically in a frame. These are deployed *in situ* for approximately two weeks (depending on programme design) and then collected for analysis and processing. A detailed overview of the use of periphyton sampling methodology, with guidance on taxonomy, can be found in Stevenson and Bahls (1999).

Figure 5.12 Collecting periphyton by gently scraping the surface of a stone removed from the stream into a container of clean water. © Deborah Chapman

REFERENCES

Alfonso, S., Gesto, M. and Sadoul, B. (2021). Temperature increase and its effects on fish stress physiology in the context of global warming. *Journal of Fish Biology*, 98(6), 1487-1589. https://doi.org/10.1111/jfb.14599

An, K-G., Park, S.S. and Shin, J-Y. (2002). An evaluation of a river health using the index of biological integrity along with relations to chemical and habitat conditions. *Environment International*, 28(5), 411-420. https://doi.org/10.1016/S0160-4120(02)00066-1

Barbour, M.T., Gerritsen, J., Snyder, B.D. and Stribling, J.B. (1999). Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C. Available at: https://www3.epa.gov/region1/npdes/merrimackstation/pdfs/ar/AR-1164.pdf

Bellinger, E.G. and Sigee, D.C. (2010). *Freshwater Algae: Identification and Use as Bioindicators*. 1st edition. John Wiley & Sons, Ltd.

Bilotta, G.S and Brazier, R.E. (2008). Understanding the influence of suspended solids on water quality and aquatic biota. Water Research, 42(12), 2849-2861. https://doi.org/10.1016/j.watres.2008.03.018

Bonacina, L., Fasano, F., Mezzanotte, V. and Fornaroli, R. (2023). Effects of water temperature on freshwater macroinvertebrates: a systematic review. *Biological Reviews*, 98(1), 191-221. https://doi.org/10.1111/brv.12903

Botts, W. (2009). An Index of Biological Integrity (IBI) for "True" Limestone Streams, PA Department of Environmental Protection, Harrisburg, Pennsylvania, USA. Available at: https://files.dep.state.pa.us/water/Drinking%20Water%20and%20Facility%20Regulation/WaterQualityPortalFiles/Technical%20Documentation/An%20Index%20of%20Biological%20Integrity-Limestone%20Streams.pdf

Boudou, A. and Ribeyre, F. (1997). Aquatic Ecotoxicology: From the Ecosystem to the Cellular and Molecular Levels. *Environmental Health Perspectives*, 105 (Supplement 1), 21-35. https://doi.org/10.1289/ehp.97105s121 Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1470317/

Brookes, J.D., Antenucci, J., Hipsey, M., Burch, M.D., Ashbolt, N.J., and Ferguson, C. (2004). Fate and transport of pathogens in lakes and reservoirs. *Environment International*, 30(5), 741-759. http://doi.org/10.1016/j.envint.2003.11.006

Carter, J.L. and Resh, V.H. (2001). After site selection and before data analysis: sampling, sorting, and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies. *Journal of the North American Benthological Society*, 20(4), 658-682. Available at: https://www.journals.uchicago.edu/doi/10.2307/1468095

Chapman, D.V., Bradley, C., Gettel, G.M., Hatvani, I.G., Hein, T., Kovacs, J. *et al.* (2016). Developments in water quality monitoring and management in large river catchments using the Danube River as an example. *Environmental Science & Policy*, 64, 141-154.

Cocco, E., Guignard, C., Hoffmann, L. and Bohn T. (2011). Rapid analysis of polychlorinated biphenyls in fish by pressurised liquid extraction with in-cell cleanup and GC-MS, International *Journal of Environmental Analytical Chemistry*, 91:4, 333-347. https://doi.org/10.1080/03067319.2010.496048

Coste M., Boutry S., Tison-Rosebery J. and Delmas F. (2009). Improvements of the Biological Diatom Index (BDI): description and efficiency of the new version (BDI-2006). Ecological. Indicators. 9, 621–650. https://doi.org/10.1016/j.ecolind.2008.06.003

Croft, P.S. (1986). *A key to the major groups of British freshwater invertebrates*. Field Studies Council, Preston Montford. Available via: https://www.field-studies-council.org/shop/publications/freshwater-invertebrates-aidgap/

Department of Environment and Science (2018). *Monitoring and Sampling Manual: Environmental Protection (Water) Policy*. Brisbane: Department of Environment and Science Government. Available at: https://environment.des.qld.gov.au/management/water/quality-guidelines/sampling-manual

Diehl, P., Gerke, T., Jeuken, A., Lowis, J., Steen, R., van Steenwijk, J. *et al.* (2005). Early Warning Strategies and Practices Along the River Rhine. *Handbook of Environmental Chemistry*, 5, 99-124. Available at: https://link.springer.com/chapter/10.1007/698_5_015

European Commission (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 establishing a framework for Community action in the field of water policy. *Official Journal of the Communities* L 327 (43), 1–73 22 December 2000.

European Commission (2005). Common Implementation Strategy for the Water Framework Directive (2000/60/EC) Guidance Document No. 13. Overall Approach to the Classification of Ecological Status and Ecological Potential. Luxembourg: Office for Official Publications of the European Communities. Available at: https://circabc.europa.eu/sd/a/06480e87-27a6-41e6-b165-0581c2b046ad/Guidance%20No%2013%20-%20Classification%20of%20Ecological%20Status%20(WG%20A).pdf

European Commission (2006). Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. *Official Journal of the European Union*, L 64, 37-51. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006L0007

European Commission (2014). Common Implementation Strategy for the Water Framework Directive (2000/60/EC). *Guidance Document No. 32 on Biota Monitoring (The Implementation of EQS$_{biota}$) under the Water Framework Directive*. Luxembourg: Office for Official Publications of the European Communities. Available at: Guidance No 32 - Biota Monitoring.pdf (europa.eu)

European Committee for Standardization (2003). Water Quality Sampling of Fish with Electricity. CEN EN 14011:2000. Brussels. European Committee for Standardization. https://standards.iteh.ai/catalog/standards/cen/cd873486-340e-4ee6-ad71-2fbdf24907cc/en-14011-2003

European Environment Agency (2016). 4.2 Biological Assessment of River Quality. Available at: https://www.eea.europa.eu/publications/92-9167-001-4/page021.html Accessed 31 August 2023.

Feeley, H.B., Davis, S., Bruen, M., Blacklocke, S. and Kelly-Quinn, M. (2012). The impact of a catastrophic storm event on benthic macroinvertebrate communities in upland headwater streams and potential implications for ecological diversity and assessment of ecological status. *Journal of Limnology*, 71(2), 309-318. https://doi.org/10.4081/jlimnol.2012.e32

Forster, B. and Pinedo, C.A. (2016). *Bacteriological Examination of Waters: Membrane Filtration Protocol*. American Society for Microbiology. Available at: https://asm.org/ASM/media/Protocol-Images/Bacteriological-Examination-of-Waters-Membrane-Filtration-Protocol.pdf?ext=.pdf Accessed: 8 September 2023.

Fowler, J., Cohen, L. and Jarvis, P. (1998). *Practical Statistics for Field Biology.* Second Edition. John Wiley and Sons, Chichester, 272 pp.

Friedrich, G., Chapman, D. and Beim, A. (1996). The Use of Biological Material. In Chapman, D.V. [ed.] *Water Quality Assessments. A Guide to Use of Biota, Sediments and Water in Environmental Monitoring.* 2nd edition. London: E and FN Spon. Available at: https://apps.who.int/iris/handle/10665/41850

Gerhardt, A., Janssens de Bisthoven, L., and Penders, E. (2003). Quality Control of Drinking Water from the River Rhine with the Multispecies Freshwater Biomonitor. *Aquatic Ecosystem Health & Management,* 6(2), 159-166. http://dx.doi.org/10.1080/14634980301466

Hawkes, H.A. (1977). Biological classification of rivers: conceptual basis and ecological validity. In: J.S. Alabaster [ed.] *Biological Monitoring of Inland Fisheries,* Applied Science Publishers Ltd, London.

Hawkes, H.A. (1998). Origin and Development of the Biological Monitoring Working Party Score System. *Water Research,* 32(3), 964-968. https://doi.org/10.1016/S0043-1354(97)00275-3

Hellawell, J.M. (1977). Biological surveillance and water quality monitoring. In: Alabaster, J.S (ed.) *Biological Monitoring of Inland Fisheries.* Applied Science Publishers Ltd., London.

Hynes, H.B.N. (1960). The Biology of Polluted Waters. Liverpool University Press, Liverpool, 202 pp.

International Organization for Standardization (2000). *ISO 8689-1:2000 Water quality — Biological classification of rivers — Part 1: Guidance on the interpretation of biological quality data from surveys of benthic macroinvertebrates.* Geneva. Available at: https://www.iso.org/standard/28368.html

International Organization for Standardization (2006a). *ISO 19458:2006. Water quality - Sampling for microbiological analysis.* Geneva. Available at: https://www.iso.org/standard/33845.html

International Organization for Standardization (2006b). *ISO 15553:2006 Water quality — Isolation and identification of Cryptosporidium oocysts and Giardia cysts from water.* Geneva. Available at: https://www.iso.org/standard/39804.html

International Organization for Standardization (2007a). *ISO 11348-3 Water quality — Determination of the inhibitory effect of water samples on the light emission of Vibrio* fischeri *(Luminescent bacteria test) — Part 3: Method using freeze-dried bacteria.* Geneva, Available at: https://www.iso.org/standard/40518.html

International Organization for Standardization (2007b). *ISO 23893-1:2007 Water quality — Biochemical and physiological measurements on fish — Part 1: Sampling of fish, handling and preservation of samples.* Geneva. Available at: https://www.iso.org/standard/40566.html

International Organization for Standardization (2012a). *ISO 8692:2012 Water quality — Fresh water algal growth inhibition test with unicellular green algae.* Geneva. Available at: https://www.iso.org/obp/ui/#iso:std:iso:8692:ed-3:v1:en

International Organization for Standardization (2012b). *ISO 6341:2012 Water quality — Determination of the inhibition of the mobility of* Daphnia magna *Straus (Cladocera, Crustacea) — Acute toxicity test.* Geneva. Available at: https://www.iso.org/obp/ui/#iso:std:iso:6341:ed-4:v1:en

International Organization for Standardization (2012c). *ISO 11350:2012. Water quality - Determination of the genotoxicity of water and wastewater* - Salmonella/*microsome fluctuation test (Ames fluctuation test).* Geneva, Available at:. https://www.iso.org/standard/50393.html

International Organization for Standardization (2012d). *ISO 9308-2:2012 Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 2: Most probable number method.* Geneva. Available at: https://www.iso.org/standard/52246.html

International Organization for Standardization (2014) *ISO 9308-1:2014 Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora.* Geneva. Available at: https://www.iso.org/standard/55832.html

International Organization for Standardization (2018). *ISO 5667-3:2018 - Water quality - Sampling - Part 3: Preservation and handling of water samples.* Geneva. Available at: https://www.iso.org/standard/72370.html

International Organization for Standardization (2023). *ISO 5667-1:2023 Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques.* Geneva. Available at: https://www.iso.org/standard/84099.html

Jaccard, P. (1901). Étude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bulletin de la Société Vaudoise des Sciences Naturelles,* 37, 547-579.

Jorge, M.B., Loro, V.L., Bianchini, A., Wood, C.M., and Gillis, P.L. (2013). Mortality, bioaccumulation and physiological responses in juvenile freshwater mussels (*Lampsilis siliquoidea*) chronically exposed to copper. *Aquatic Toxicology,* 126, 137-147.

Karr, J.R. (1981). Assessment of biotic integrity using fish communities. *Fisheries*, 6, 21-27.

Karr, J.R., Fausch, K.D., Angermeier, P.L., Tant, P.R., and Schlosser, I.J. (1986). Assessing Biological Integrity in Running Waters A Method and Its Rationale. *Illinois Natural History Survey Special Publication* 5, 1-28.

Kay, D., Wyer, M., Crowther, J., Stapleton, C., Bradford, M., McDonald, A. *et al.* (2005). Predicting faecal indicator fluxes using digital land use data in the UK's sentinel Water Framework Directive catchment: The Ribble study. *Water Research*, 39, 3967-3981.

Kolarević, S., Kračun-Kolarević, M., Kostić, J., Slobodnik, J., Liška, I., Gačić, Z. *et al.* (2016). Assessment of the genotoxic potential along the Danube River by application of the comet assay on haemocytes of freshwater mussels: The Joint Danube Survey 3. *Science of the Total Environment,* 540, 377-85. https://doi.org/10.1016/j.scitotenv.2015.06.061

Kramer, K.J.M. and Foekema, E.M. (2001). The "Musselmonitor®" as Biological Early Warning System. In: Butterworth, F.M., Gunatilaka, A., Gonsebatt, M.E. (eds) *Biomonitors and Biomarkers as Indicators of Environmental Change 2.* Environmental Science Research, vol 56. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-1305-6_4

Kumar, K.S., Dahms, H.U., Lee, J.S., Kim, H.C., Lee, W.C., and Shin, K.H. (2014). Algal photosynthetic responses to toxic metals and herbicides assessed by chlorophyll α fluorescence. *Ecotoxicology and Environmental Safety,* 104, 51-71.

Lavoie I., Campeau S., Zugic-Drakulic N., Winter J. G. and Fortin C. (2014). Using diatoms to monitor stream biological integrity in Eastern Canada: an overview of 10 years of index development and ongoing challenges. *Science of the Total Environment,* 475, 187–200. https://doi.org/10.1016/j.scitotenv.2013.04.092

Lobo, E.A., Heinrich, C.G., Schuch, M., Wetzel, C.E. and Ector, L. (2016). Diatoms as Bioindicators in Rivers. In: Necchi Jr, O. (ed) *River Algae*. Springer, Cham. https://doi.org/10.1007/978-3-319-31984-1_11

Lv, X., Lu, Y., Yang, X., Dong, X., Ma, K., Xiao, S. *et al.* (2015). Mutagenicity of drinking water sampled from the Yangtze River and Hanshui River (Wuhan section) and correlations with water quality parameters. *Scientific Reports*, 5, 9572 https://doi.org/10.1038/srep09572

Madsen, J. (1999). Point intercept and line intercept methods for aquatic plant management. APCRP Technical Notes Collection (TN APCRP-M1-02). US Army Engineer Research and Development Center, Vicksburg, MS, USA, pp 16. Available at: https://apps.dtic.mil/sti/pdfs/ADA361270.pdf

Madsen, J.D. and Wersal, R.M. (2017). A review of aquatic plant monitoring and assessment methods. *Journal of Aquatic Plant Management,* 55, 1-12. Available at: https://www.apms.org/wp-content/uploads/japm-55-01-01.pdf

Manly, B.F.J. and Navarro Alberto, J.A. (2015). *Introduction to Ecological Sampling.* CRC Press, Boca Raton, FL. 228 pp.

Margot, J., Rossi, L., Barry, D.A. and Holliger, C. (2015). A review of the fate of micropollutants in wastewater treatment plants. *WIREs Water,* 2(5), 457-487. https://doi.org/10.1002/wat2.1090

Masouras, A., Karaouzas, I., Dimitriou, E., Tsirtsis, G. and Smeti, E. (2021). Benthic Diatoms in River Biomonitoring—Present and Future Perspectives within the Water Framework Directive. *Water*, 13, 478. https://doi.org/10.3390/w13040478

Mebane, C.A., Maret, T.R. and Hughes, R.M. (2003). An Index of Biological Integrity (IBI) for Pacific Northwest Rivers. *Transactions of the American Fisheries Society,* 132, 239–261. https://doi.org/10.1577/1548-8659(2003)132%3C0239:AIOBII%3E2.0.CO;2

Meyer, J.L., Strayer, D.L., Wallace, J.B., Eggert, S.L., Helfman, G.S. and Leonard, N.E. (2007). The Contribution of Headwater Streams to Biodiversity in River Networks. *Journal of the American Water Resources Association (JAWRA),* 43(1), 86-103. Available at: https://www.srs.fs.usda.gov/pubs/ja/ja_meyer002.pdf

Organisation for Economic Co-operation and Development (1987). *The use of Biological Tests for Water Pollution Assessment and Control.* Environment Monographs No. 11. Paris, 70 pp.

Paisley, M.F., Trigg, D.J., and Walley, W.J. (2007). Revision and Testing of BMWP scores. Final report SNIFFER Project WFD72a. Edinburgh, SNIFFER. Available at: https://www.sniffer.org.uk/Handlers/Download.ashx?IDMF=49d44450-1746-40d8-8c08-f8e97bc0aeea

Parsons, J. (2001). *Aquatic Plant Sampling Protocols.* Publication No. 01-03-017, Washington State Department of Ecology Publications Distributions Office, Olympia WA. Available at: https://apps.ecology.wa.gov/publications/documents/0103017.pdf

Pielou, E. (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology,* 13, 131-144. https://doi.org/10.1016/0022-5193(66)90013-0

Quilliam, R.S., Clements, K., Duce, C., Cottrill, S.B., Malhah, S.K. and Jones, D.L. (2011). Spatial variation of waterborne *Escherichia coli* – implications for routine water quality monitoring. *Journal of Water and Health,* 9, 734–737. https://doi.org/10.2166/wh.2011.057

Ralf, P.J., Smith, R.A., Macinnis-Ng, C.M.O. and Seery, C.R. (2007). Use of fluorescence-based ecotoxicological bioassays in monitoring toxicants and pollution in aquatic systems: Review. *Toxicological & Environmental Chemistry,* 89(4), 589-607. https://doi.org/10.1080/02772240701561593

Ranasinghe, P., Weerasinghe, S. and Kaumal, M.N. (2016). Determination of Heavy Metals in Tilapia using Various Digestion Methods. *International Journal of Scientific Research and Innovative Technology,* 3(6), 2313–3759. Available at: https://www.researchgate.net/publication/325450561_Determination_of_Heavy_Metals_in_Tilapia_using_Various_Digestion_Methods Accessed 8 September 2023.

Scudder, B.C., Chasar, L.C., DeWeese, L.R., Brigham, M.E., Wentz, D.A., and Brumbaugh, W.G. (2008). Procedures for collecting and processing aquatic invertebrates and fish for analysis of mercury as part of the National Water-Quality Assessment Program. U.S. Geological Survey Open-File Report 2008–1208, pp. 34. Available at: https://pubs.usgs.gov/of/2008/1208/pdf/ofr20081208.pdf

Shahjahan, M., Taslima, K., Rahman, M.S., Al-Emran, M., Alam, S.I. and Faggio, C. (2022). Effects of heavy metals on fish physiology – A review. *Chemosphere,* 300, 134519, https://doi.org/10.1016/j.chemosphere.2022.134519

Shannon, C.E., and Weaver, W. (1949). *The Mathematical Theory of Communication.* University of Illinois Press, Urbana. Available at: https://www.press.uillinois.edu/books/?id=p725487

Simpson E.H. (1949). Measurement of diversity. Nature, 163, 688. Available at: https://www.nature.com/articles/163688a0

Sinche, F., Cabrera, M., Vaca, L., Segura, E. and Carrera, P. (2022). Determination of the ecological water quality in the Orienco stream using benthic macroinvertebrates in the Northern Ecuadorian Amazon. *Integrated Environmental Assessment and Management,* 19(3), 615–625. https://doi.org/10.1002/ieam.4666

Smith, B., Storey, R. and Valois, A. (2019). *Benthic Macroinvertebrate Field Identification Guide.* NIWA. Available at: Benthic Macroinvertebrates_Field ID Guide_MT2019.indd (niwa.co.nz)

Standard Methods Committee of the American Public Health Association, American Water Works Association, and Water Environment Federation. (2023). *Standard Methods for the Examination of Water and Wastewater.* 24th edition. Lipps, W.C., Baxter, T.E., Braun-Howland, E. (eds) Washington DC: APHA Press. Available at: https://www.standardmethods.org/about/

Stevenson, R.J. and Bahls, L.L. (1999). Chapter 6: Periphyton Protocols. In: Barbour, M.T., Gerritsen, J., Snyder, B.D., and Stribling, J.B. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish,* Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C. https://www3.epa.gov/region1/npdes/merrimackstation/pdfs/ar/AR-1164.pdf

Titus, J.E. (1993). Submersed Macrophyte Vegetation and Distribution Within Lakes: Line Transect Sampling. *Lake and Reservoir Management,* 7(2), 155-164. https://doi.org/10.1080/07438149309354267

Toner, P., Bowman, J., Clabby, K., Lucey, J., McGarrigle, M., Concannon, C., Clenaghan, C., Cunningham, P., Delaney, J., O'Boyle, S., MacCarthaigh, M., Craig, M. and Quinn, R. (2005). *Water Quality in Ireland 2001-2003.* Environmental Protection Agency, Johnstown Castle.

Tornevi, A., Bergstedt, O. and Forsberg, B. (2014). Precipitation Effects on Microbial Pollution in a River: Lag Structures and Seasonal Effect Modification. *PLoS ONE* 9(5), e98546 https://doi.org/10.1371/journal.pone.0098546

United States Environmental Protection Agency (2000). *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Vol 1 Fish Sampling and Analysis.* EPA 823-B-00-007. Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2015-06/documents/volume1.pdf

United States Geological Survey (2002). Assessment of Stream Quality Using Biological Indices at Selected Sites in the Delaware River Basin, Chester County, Pennsylvania, 1981-97. USGS Fact Sheet FS-116-02. New Cumberland, PA. 4 pp. Available at: https://pubs.usgs.gov/fs/2002/0116/fs20020116.pdf

Vadas Jr, R.L., Hughes, R.M., Bae, Y.J., Baek, M.J., Bello Gonzáles, O.C., Callisto, M. *et al.* (2022). Assemblage-based biomonitoring of freshwater ecosystem health via multimetric indices: A critical review and suggestions for improving their applicability. *Water Biology and Security,* 1(3), 100054, https://doi.org/10.1016/j.watbs.2022.100054 .

Walley, W.J. and Hawkes, H.A. (1996). A computer-based reappraisal of the Biological Monitoring Working Party scores using data from the 1990 river quality survey of England and Wales. *Water Research,* 30(9): 2086–2094. https://doi.org/10.1016/0043-1354(96)00013-9

Walley, W.J. and Hawkes, H.A. (1997). A computer-based development of the Biological Monitoring Working Party score system incorporating abundance rating, site type and indicator value. *Water Research,* 31(2), 201–210. https://doi.org/10.1016/S0043-1354(96)00249-7

Washington, H.G. (1984). Diversity, biotic and similarity indices. A review with special relevance to aquatic systems. *Water Research,* 18(6), 653–694. https://doi.org/10.1016/0043-1354(84)90164-7

Weigel, B.M., Henne, L.J. and Martinez-Rivera, L.M. (2002). Macroinvertebrate-based index of biotic integrity for protection of streams in west-central Mexico. *Journal of the North American Benthological Society,* 21(4), 686–700. Available at: https://www.academia.edu/77532808/Macroinvertebrate_based_index_of_biotic_integrity_for_protection_of_streams_in_west_central_Mexico

Wlodkowic D, Karpiński TM. (2021). Live-Cell Systems in Real-Time Biomonitoring of Water Pollution: Practical Considerations and Future Perspectives. Sensors (Basel), 21(21), 7028. https://doi.org/10.3390/s21217028

World Health Organization (2022). *Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda.* 614 pp. Geneva. Available at: https://www.who.int/publications/i/item/9789240045064

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