Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes
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Chapter 11 - BIOLOGICAL MONITORING

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Water quality can be described in terms of physical, chemical and biological characteristics (see Chapter 2). Although biologists have been studying the effects of human activities on aquatic systems and organisms for decades, their findings have only relatively recently been translated into methods suitable for monitoring the quality of water bodies. Artificial (and in some cases natural) changes in the physical and chemical nature of freshwaters can produce diverse biological effects ranging from the severe (such as a total fish kill) to the subtle (for example changes in enzyme levels or sub-cellular components of organisms). Changes like these indicate that the ecosystem and its associated organisms are under stress or that the ecosystem has become unbalanced. As a result there could be possible implications for the intended uses of the water and even possible risks to human health.

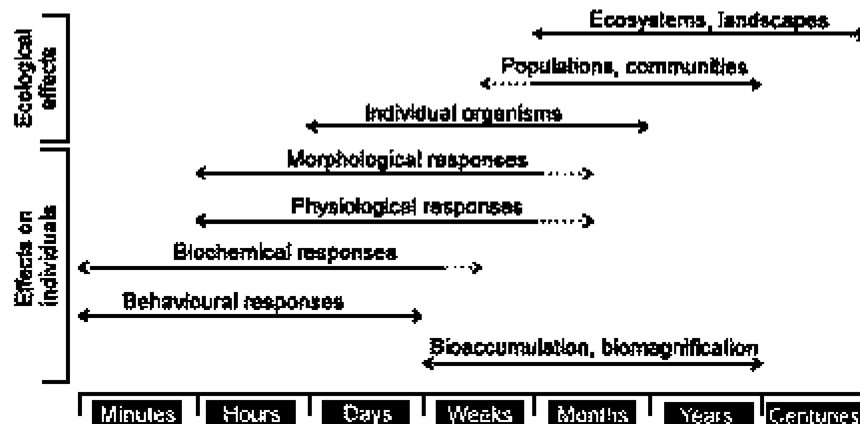
The responses of biological communities, or of the individual organisms, can be monitored in a variety of ways to indicate effects on the ecosystem. The co-existence and abundance of certain species at particular locations can indicate, for example, whether that habitat has been adversely altered. The reactions of individual organisms, such as behavioural, physiological or morphological changes, can also be studied as responses to stress or adverse stimuli (for example caused by the presence of contaminants). Some approaches are suitable for field use and some have been developed specifically for use in the laboratory (particularly toxicity tests and bioassays).

Certain contaminants, particularly metals and organic compounds, may be accumulated in the tissues of organisms. Therefore, chemical analysis of the appropriate biological tissues can be used to show that the organism has been exposed to contaminants and, in some cases, to monitor the spatial distribution, or accumulation, of that contaminant in the aquatic ecosystem.

When designing a monitoring programme biological methods should be considered along with other approaches (see Chapter 3). They should be used only if appropriate to the objectives of the programme and should always be accompanied by the appropriate physical and chemical measurements that are necessary for proper interpretation of results. Biological monitoring should not be seen as an alternative to physical and chemical monitoring but as a useful complementary approach. Although physical and chemical analyses can identify that many contaminants may be present, biological methods can integrate responses to combinations of all contaminants and to other sources of environmental stress, thereby indicating overall effects in a water body. Physical and chemical analyses give a measurement which is valid only for the instance in time when the sample was collected, whereas some biological methods reflect the effects of the physical and chemical conditions to which the organisms were exposed over a period of time (see Figure 11.1). Many

biological approaches can be cheaper than chemical methods in terms of equipment, but would normally place heavy demands on field and laboratory personnel. Financial savings can sometimes be made in a monitoring programme by using biological methods to "trigger" the need for intensive and sensitive chemical analyses (see section 11.5).

Figure 11.1 The duration of environmental effects which can be monitored by different biological approaches (Modified from de Zwart, 1995)



A disadvantage of biological methods is that it can be difficult to relate observed effects to specific aspects of environmental disturbance, such as contamination or natural changes. For example, methods do not always provide precise information on the identity of a contaminant unless supplementary information from chemical analyses is available. In addition, the response of organisms may be affected by their natural cycles, such as life stage and reproductive condition. Consequently, like other techniques, biological monitoring methods should be developed and interpreted by experienced biologists.

Within the context of this chapter it is not possible to describe all biological methods which may be useful in detail. Emphasis is placed here on describing standardised methods or methods which are less costly or can be relatively easily developed for use in local circumstances. Although the methods are described principally with respect to their use for monitoring water quality for intended uses, or in relation to actual or anticipated effects, some methods (particularly bioassay techniques) can also be applied to monitoring liquid effluents prior to their discharge.

Examples of the application of some popular methods in monitoring and assessment programmes, together with techniques for the interpretation of results are given in the companion guidebook, *Water Quality Assessments*, and in other literature describing general, specific and standardised methods (see section 11.9 for some key references and widely available texts).

11.1 Selection of appropriate methods and organisms

The decision to use a biological method will be based on a number of factors:

- The objectives of the monitoring programme.
- The availability of a suitable method in relation to the objectives.
- The financial and personnel resources available.

The selection of the most suitable technique or approach is very important if objectives are to be met and the value of the biological method is to be realised. There are several important factors which must be considered during the selection process. The ability of any biological

method to indicate the state of the environment is dependent on the degree and duration of exposure of the disturbance being matched with the sensitivity and response rate of the biological process (Figure 11.1). A trained biologist is normally necessary to select and develop biological methods and to interpret their results.

The most widely applied biological method is the monitoring of bacteria associated with faecal contamination. This approach is used to monitor a very specific water quality issue and gives a direct indication of risk to human health. Microbiological methods are treated in detail in Chapter 10.

Assessments of long-term water quality variations on a regional basis often include biological methods as a means of indicating the overall condition of ecosystems. Baseline surveys of aquatic habitats and their communities provide a reference point against which future monitoring programme results can be evaluated. Specific objectives, such as early-warning of environmental degradation close to an important drinking water intake, require sensitive biological methods which give clear, unambiguous results, immediately the adverse environmental conditions occur. Where existing standardised methods are not available it may be necessary to adapt a technique to local conditions, using organisms found naturally in the aquatic habitat to be monitored.

If the financial resources and the technical capacity for extensive chemical monitoring are limited, it may be desirable to use a biological method in order to determine the presence and severity of an environmental effect or to trigger the need for more complex techniques which are not used routinely. Nevertheless, it should always be borne in mind that meaningful interpretation of the results of biological methods usually requires some basic physical and chemical measurements in order to help the biologist to identify natural influences on aquatic organisms and communities.

11.1.1 Selection of techniques

Once it has been established that biological methods will provide useful information in a monitoring programme, an appropriate technique must be selected from the many methods available. The principal biological methods can be divided into five categories:

- Ecological methods: based on community structure and presence or absence of species.

- Physiological and biochemical methods: based on community metabolism (such as oxygen production or consumption, growth rates) or biochemical effects in individuals or communities (such as enzyme inhibition).

- Controlled biotests: based on measuring toxic (or beneficial) effects (death, growth rates, reproductive capacity) on organisms under defined laboratory conditions or the effects on behaviour *in situ* or in controlled environments.

- Contaminants in biological tissues: based on measurements of the accumulation of specific contaminants in the tissues of organisms living in the environment (passive monitoring) or deliberately exposed in the environment (active monitoring).

- Histological and morphological methods: based on the observation of cellular changes or morphological changes such as gill damage or skin lesions in fish.

Table 11.1 Examples of biological monitoring methods which can be applied in freshwaters

Biological approach	Organisms or methods used	Organism response or observation criterion
Single species, acute toxicity tests in the laboratory	Fish *Daphnia* Bacterial luminescence Daphnia IQ test Rotoxkit F Thamnotoxkit F Toxichromotest Ames test, SOS chromotest, Mutatox test	Death Death, immobilisation Light emission Enzyme inhibition Death Death Enzyme inhibition Bacterial mutagenicity
Single species, sub-lethal toxicity tests in the laboratory	Protozoa/bacteria Algae *Daphnia* Fish	Population growth Population growth Reproduction Early life stage, growth, chromosome aberration
	Lemna test	Colony growth
Sub-organismal toxicity test in the laboratory	*In vitro* tissue test	Growth, death histopathology
Early warning or semi-continuous field toxicity tests	Fish	Ventilation Rheotaxis Swimming behaviour
	Algae Bacteria	Productivity Luminescence Respiration
	Daphnia Mussels	Swimming activity Valve movement
Field toxicity test (active monitoring)	Caged organisms	Death, growth, reproduction, bioaccumulation, scope for growth Biomarkers: metallothioneine formation, lysosome stability, mixed function oxidase (MFO) induction
Observation of effects in the field (passive monitoring)	Eco-epidemiology in selected species: fish, *Chironomus* Indicator species Colonisation of artificial substrates Community structure: benthicmacrofauna, diatoms Ecological function	Incidence of diseases and morphological variations Presence or absence Species composition, diversity abundance Species composition, diversity abundance Primary productivity, respiration, biomass, turnover, degradation, material cycling

Source: After de Zwart, 1995

Most of the approaches listed above have one or more specific methods which are widely accepted as useful and practicable for environmental monitoring (Table 11.1).

The global diversity of aquatic species has led to the adaptation and modification of several standard biological methods (see section 11.5), for use with an enormous range of organisms. Many of these methods are useful for indicating adverse effects in aquatic environments, but often only in specific situations. It has been estimated that there are about

120 different laboratory-based toxicity tests, and about 100 different field methods for assessing aquatic community effects, described in the international literature (de Zwart, 1995). The suitability of any particular method must be evaluated with respect to:

- the information it will generate (ecological as well as in relation to environmental problems),

- the specificity of the method and the selected organisms,

- the ability of the habitat or the organisms to return to their natural condition (i.e. recolonise, regenerate) once the environmental problem is removed,

- the sensitivity and rate and range of responses of the organisms or methods in relation to the environmental stress,

- the cost and practicality of the method, and the ability of the method to provide information that can be translated into useful management information and to help achieve the monitoring programme objectives.

As with all other components of a monitoring programme, it is important to assess the suitability of biological methods against the specified objectives of the whole programme and not only against the biological component of the programme.

The effects demonstrated by organisms experiencing environmental stress range from death or migration from the affected area to subtle biochemical changes at the sub-cellular level. Many of these effects have been incorporated and developed into possible monitoring methods, but most are only applicable to certain types of environmental stress, to particular time scales of stress effects or to specific habitats or localities.

The most widely applicable methods (i.e. geographically) are those which are laboratory based, using samples of water collected in the field, such as toxicity tests and bioassays. However, the direct relevance of such methods to the field situation is sometimes questioned and not always clear. Conversely, field-based methods which integrate and accumulate direct effects in the water body or aquatic habitat are usually very specific to geographic regions and sometimes to specific aquatic habitats within those regions. Care must be taken to apply these methods only in appropriate locations (unless they have been specifically adapted) and to choose monitoring sites which are directly comparable with each other.

11.1.2 Choice of organisms

In order to detect environmental disturbance using biota it is important that the organisms reflect the situation at the site from which they are collected, i.e. they must not migrate. As a result, the most widely used group of aquatic organisms are the benthic macroinvertebrates. These organisms are usually relatively immobile, thereby indicating local conditions and, since many have life spans covering a year or more, they are also good integrators of environmental conditions. They are ubiquitous and abundant in aquatic ecosystems and relatively easy to collect and identify (at least to family level). No expensive equipment is necessary for their collection. It is sometimes possible to get a qualitative impression of water quality in the field because the presence or absence of certain groups is related to their tolerance to specific environmental conditions (such as organic pollution) and may be well documented (Figure 11.2).

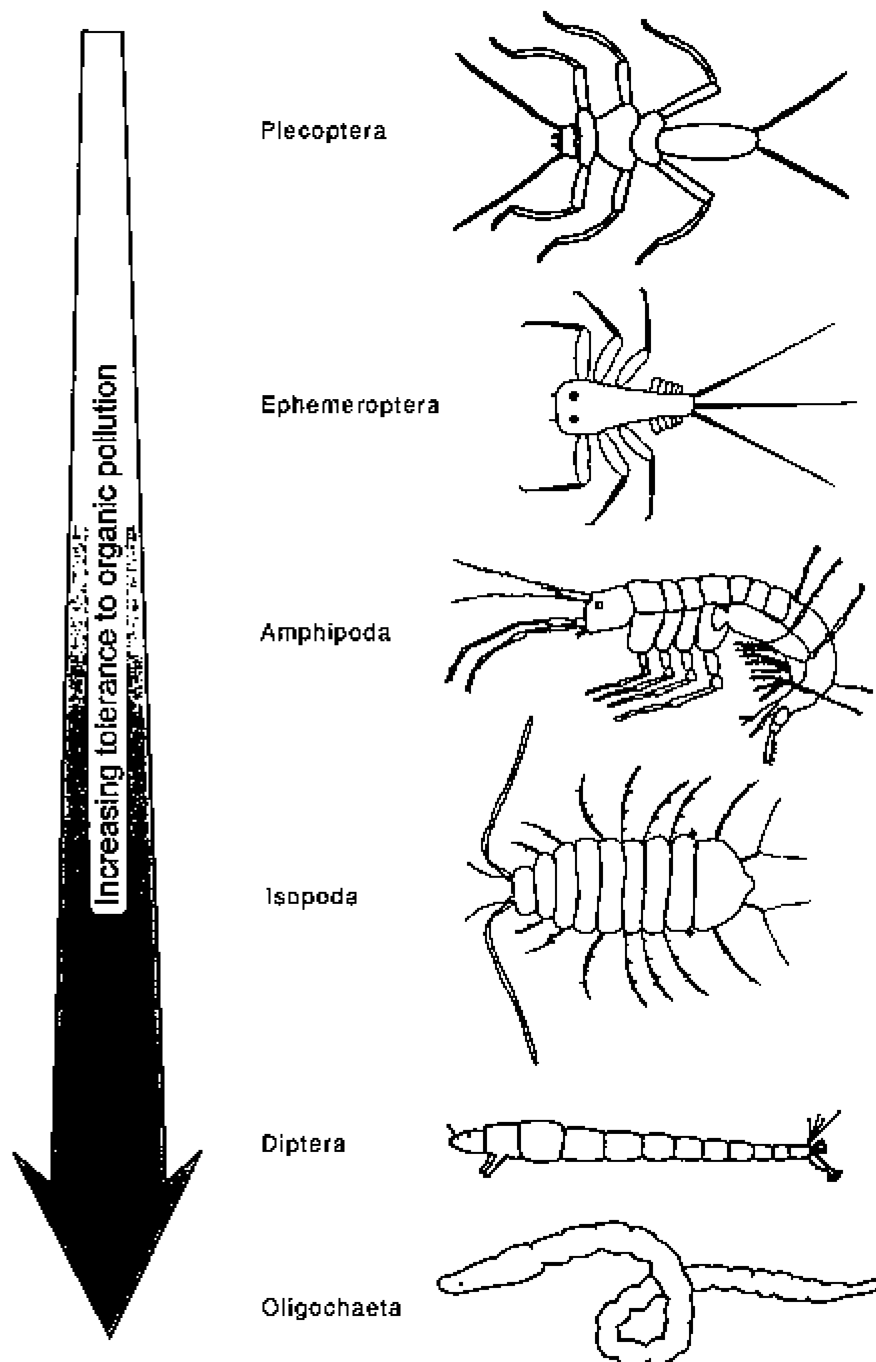
For evaluation of long-term (i.e. several years) environmental conditions over a broader area, such as a river stretch or small lake, fish have several advantages. They are long lived and

mobile and, because different species cover the full range of trophic levels (omnivores, herbivores, insectivores, planktivores and piscivores), fish community structure is a good indicator of overall environmental condition. Fish also have the advantage that they are an important source of food for humans which gives them a direct relevance for assessing potential contamination. They are also important for recreational and commercial activities, and monitoring them gives an evaluation of water quality which is readily understood by non-specialists. In addition, fish often form the basis of ecological water quality standards. Basic sampling methods can be cheap and easy (such as netting techniques) and a trained fish biologist can make a field evaluation of the condition of the habitat without harming the fish.

The other group of organisms which has been fairly widely used for water quality monitoring is the algae. They are widely used in the evaluation of eutrophication and turbidity. Algae are primary producers and are, therefore, mostly affected by physical and chemical variations in their environment. Their pollution tolerances are also fairly well documented. Simple, non-taxonomic methods are well developed, such as the determination of chlorophyll (see sections 11.3 and 7.9), and sampling methods are simple and cheap. Although other groups of organisms have been found to be suitable for particular biological methods they are less suitable for indicating the integrated effects of environmental disturbance.

The following sections present details of the basic procedures for a few selected methods which are widely applicable, relatively less expensive to perform and most easily adapted to local conditions. Examples and results of using some biological methods, together with the manipulation and interpretation of the resultant data, are given in the companion guidebook *Water Quality Assessments*.

Figure 11.2 The relative tolerance to organic pollution of some key groups of aquatic macroinvertebrates (Adapted from Mason, 1981)



11.2 Ecological methods

Each aquatic organism has particular requirements with respect to the physical, chemical and biological condition of its habitat. Changes in these conditions can result in reduction in species numbers, a change in species dominance or total loss of sensitive species by death or migration. The presence or absence of certain species in relation to particular water quality characteristics has been exploited in the development of ecological methods based on “indicator species”. These methods are frequently referred to as biotic indices and require a good knowledge of the organisms in the specific environments to which the methods are applied. Information on the physical and chemical status of the aquatic habitats in which

these methods are used is also essential in order to determine whether certain species could survive there, even under undisturbed conditions. The fluctuations in diversity and numerical abundance of species have also been developed into a variety of community structure indices. These methods often require a less detailed knowledge of the species in a particular habitat and have, as a result, been rather widely applied without adequate investigation into their biological relevance. They are, nevertheless, very useful while a (possibly) more sensitive method is being developed or tested. Macroinvertebrates are particularly suitable for both of these approaches.

At the most basic level, ecological monitoring involves determining the species and abundance of the fauna and flora present and observing any changes in the community structure over time. However, other methods have been developed which present the information such that non-biologists can interpret the observed differences between sampling sites. These approaches produce numerical indices in which the magnitude of the index value is related to a qualitative assessment of ecosystem or water quality (e.g. from clean to polluted). Unfortunately, such simplified interpretations are open to mis-use and the importance of interpretation of the results by a trained biologist cannot be over-emphasised.

A preliminary survey or sampling programme, using the proposed methods at the intended sites, can help to eliminate possible problems and to save time and effort during the main programme. The survey can evaluate the most suitable organisms, test the efficiency of the sampling device and determine the size and number of samples required in relation to the size, density and spatial distribution of the organisms being sampled.

11.2.1 Indicator organisms

The most studied and monitored degradation of water quality is due to organic pollution arising from sewage discharges. In northern temperate regions the typical assemblages of organisms associated with a certain degree of organic pollution (or the gradual recovery from the effects of an organic discharge) are well documented and numerous biotic indices have been developed. In Central Europe, the association of particular (easily identified) organisms from all trophic levels with defined physical and chemical characteristics of the water, have been combined with the abundance of the organisms to calculate their saprobic value (a number between 1 and 20 representing the association of the organisms with clean to severely polluted conditions). The saprobic value is then combined with index values related to the abundance and sensitivity of the organisms to calculate the Saprobic Index (ranging from 1.0 to 4.0, indicating very clean to severely polluted waters). This index was first devised at the beginning of the twentieth century and, although it has been improved and standardised, it is time consuming and costly to conduct frequently or intensively.

Many simplified biotic indices are based on the Trent Biotic Index which was originally devised in 1964 for use in the River Trent in England. Standardised collection methods (see section 11.2.3) are used for benthic macroinvertebrates and the indices are derived from scores allocated according to the presence or absence of indicator groups and/or indicator species. The Trent Biotic Index is based on the number of groups (specified taxa) in relation to the presence of six key organisms found at the sample site. This type of simplified index is often most suited to fast flowing, upland stretches of rivers. In order to use or develop this basic approach for other geographic areas it is necessary to have detailed knowledge of the distribution of local species in relation to different environmental conditions (e.g. clean and organically contaminated). It is important that biotic indices are used together with all other available information to ensure correct interpretation of the biological information.

Both of the methods mentioned above are described in more detail in the companion guidebook *Water Quality Assessments*.

The Biological Monitoring Working Party (BMWP) score

In order to reduce the effort and taxonomic expertise necessary for routine biological assessments based on indicator organisms, there is much interest in developing score systems which rely only on identification to the family level and which are not specific to any single river catchment or geographical area. One such method, the Biological Monitoring Working Party score (BMWP) has been standardised by the International Organization for Standardization (ISO). It can be used to reflect the impact of organic pollution, such as results from sewage disposal or farm waste.

Table 11.2 The biological scores allocated to groups of organisms by the Biological Monitoring Working Party (BMWP) score

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

Groups in brackets are new groups of organisms that were previously contained in the group immediately before it in the list. These new groups are the result of developments in the taxonomic system since the BMWP score was originally prepared.

Principle: Invertebrates are collected from different habitats (e.g. gravel, silt, weed beds) at representative sites on river stretches and identified to the required taxonomic level (normally family level). Each group or family is allocated a score between 1 and 10, according to their sensitivity to environmental disturbance (Table 11.2). The most sensitive organisms, such as

stoneflies, score 10 and the least sensitive, such as oligochaete worms, score 1. The scores for each family represented in the sample are then summed to give the BMWP score. In order to reduce the effects of sample size, sampling effort and sampling efficiency on the results obtained by this method, the Average Score Per Taxon (ASPT) should also be taken into consideration. This is obtained by dividing the BMWP score by the total number of taxa (families) in the sample. The number of taxa present is indicative of the diversity of the community (see below). A BMWP score greater than 100, together with an ASPT value greater that 4, generally indicates good water quality.

Procedure

1. Use a standardised collection technique (normally a pond net, although dredges or grabs can be used in deeper water, see section 11.2.3) to collect macroinvertebrates from each of the major habitat types at the sample site (e.g. gravel, silt, weed beds). If using a pond net collect organisms for a fixed period of time, or if using a dredge or grab collect a standard volume of substrate.

Note: Each habitat type represents a part of the complete sample for the site.

2. Remove large pieces of organic debris or stones from the sample and empty it into a suitable, labelled, container.

3. If sorting and identification cannot be carried out in the field, the sample may be preserved with formaldehyde or alcohol (see Table 11.3) for transportation and storage at the laboratory.

4. Empty the sample into a white tray and sort the macroinvertebrates present into the groups identified in Table 11.2.

5. Tick off the groups present on a sample record sheet. Note that even if more than one species occurs for a particular group that group is only recorded once.

6. Add the scores for all groups ticked on the record sheet to give the BMWP score (e.g.if Oligochaeta, Assellidae, Sphaeriidae and Baetidae were present the score would be 11).

7. Add up the total number of groups occurring in the sample (for the example given in step 6 above the total number is 4).

8. Divide the BMWP score by the total number of groups present to give the ASPT (for the example above 11/4 = 2.75)

9. Record the result as BMWP..... ASPT..... (for the example above the result of BMWP 11 ASPT 2.75 would suggest very poor water quality).

11.2.2 Community structure

Community structure methods are based on the numerical abundance of each species rather than relying on particular indicator species. Some of the resultant indices are derived from mathematical principles, such as information theory, and their direct relevance to, and suitability for, the environmental situations in which they may be used should be thoroughly tested. Although a knowledge of taxonomy is required to sort and count samples of organisms, the indices can be useful to non-specialists as an indicator of whether environmental conditions are changing. Community structure indices should only be used to

study changes at the same sites over time or to compare sites with similar natural physical and chemical features. They are normally applied to samples of organisms of the same type, e.g. benthic macroinvertebrates, diatoms or fish.

Table 11.3 Selected techniques for the preservation of biological samples

Organisms	Container type	Preservation method	Storage of preserved samples
Identification and counting			
Benthic macro invertebrates	P or G	10 % Ethanol. 5 % formaldehyde	1 year
Fish	P or G	Addition of 10 % (m/m) formaldehyde. 3 g of sodium borate decahydrate and 60 ml of glycerol per litre	Short as possible but < 1 year
Macrophytes	P or OG	Addition of 5 % (m/m) formaldehyde	1 year
Periphyton	P or OG	Addition of 5 % (m/m) neutral formaldehyde	6 months in the dark
Phytoplankton	P or OG	Addition of 5 % (m/m) neutral formaldehyde or mentholate Lugol’s iodine solution	6 months in the dark
Zooplankton	P or G	Addition of 5 % (m/m) formaldehyde Lugol’s solution	1 year
Fresh weight			
Fish	P or G	Not applicable	Not applicable Weigh on site
All other groups	P or G	Cool to 2-5°C	24 hours
Dry or ash weight			
All groups	P or G	Fitter (where appropriate) Freeze to -20 *C	6 months
Chlorophyll analyses			
Phytoplankton	P	Filter immediately and add 0.2 ml of $MgCO_3$ suspension (1.0 $MgCO_3$ in 100 ml water) as last of sample is being filtered Freeze filters to -20 °C	In the dark. Minimum possible but up to a few weeks if frozen and desiccated
Chemical analysis			
Animal or plant tissue[1]		Freeze to -20 °C	1 year

A preservation method should be chosen which is suitable for all the determinations which may be necessary on a single sample. Ideally, all analyses should be carried as soon as possible after sample collection, and preservation should be avoided where feasible.

G Glass

OG Opaque glass

[1] Storage and transport containers depend on the analyses to be performed on the tissues.

For most metal analyses new polythene bags are suitable

P Potyethylene

Source: After WMO, 1988.

Diversity indices

Diversity indices are best applied to situations of toxic or physical pollution which impose general stress on the organisms. Stable ecosystems are generally characterised by a high species diversity, with each species represented by relatively few individuals. Although diversity can be reduced by anthropogenic disturbance or stress, some natural conditions can also lead to reduced diversity (such as nutrient poor headwaters) and it is very important that diversity indices are only used to compare sites of similar physical and chemical characteristics. A widely used diversity index is the Shannon Index which combines data on species or taxa richness with data on individual abundance. The species number indicates the diversity of the system and the distribution of numbers of individuals between species indicates the evenness.

Procedure for Shannon Index

1. Sort organisms into particular taxa (species, genus or family). All organisms should be identified to the same taxonomic level, e.g. genus level.

2. Count organisms in each taxonomic group.

3. Total the number of organisms in the whole sample.

4. Calculate the Shannon Index *H´* from the following formula:

$$H' = \sum_{i=1}^{s} \frac{n_i}{n} \ln \frac{n_i}{n}$$

where

s = the number of taxa in the sample
n_i = the number of individuals in the ith taxa
n = the total number of individuals in the sample

The numerical values generated cannot be taken to be indicative of any particular water quality unless extensively tested and related to physical, chemical and biological conditions in the specific water bodies from which they have been obtained (see example in Figure 14.2). They can be used, however, to show relative differences from one site to another within the same aquatic system or at the same site over time.

11.2.3 Sampling methods and sample handling

Some common sampling methods for different aquatic organisms are compared in Table 11.4. Ecological methods can use a wide range of sampling techniques ranging from qualitative collection (such as selection of macrophytes by hand), to semi-quantitative methods (such as collection of benthic organisms using a standardised handnet technique), to fully quantitative techniques (such as bottle samples for plankton or grab samples for

benthic organisms). Ecological methods based on biotic or community structure indices require the use of quantitative or semi-quantitative methods.

The simplest and cheapest method of collecting benthic invertebrates in shallow, flowing waters is by means of a standard handnet (Figure 11.3) as described below.

Table 11.4 Comparison of sampling methods for aquatic organisms

Sampler/Sampling mechanism	Most suitable Organisms	Most suitable Habitats	Advantages	Disadvantages
Collection by hand	Macrophytes, attached or clinging organisms	River and lake margins, shallow waters, stony substrates	Cheap - no equipment necessary	Qualitative only. Some organisms lost during disturbance. Specific organisms only collected
Hand net on pole (c. 500 mm mesh)	Benthic invertebrates	Shallow river beds, lake shores	Cheap, simple	Semi-quantitative. Mobile organisms may avoid net
Plankton net	Phytoplankton (c. 60 mm mesh), zoo plankton	Open waters, mainly lakes	Cheap and simple. High density of organisms per sample. Large volume or integrated samples possible	Qualitative only (unless calibrated with a flow meter). Selective according to mesh size. Some damage to organisms possible
Bottle samples (e.g. Friedinger, Van Dom, Ruttner)	Phytoplankton, zooplankton (inc. protozoa), micro-organisms	Open waters, groundwaters	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
Water pump	Phytoplankton, zooplankton (Inc. protozoa), micro-organisms	Open waters, groundwaters	Quantitative if calibrated. Rapid collection of large volume samples. Integrated depth sampling possible	Expensive and may need power supply. Sample may need filtration or centrifugation to concentrate organisms. Some damage to organisms possible.
Grab (e.g. Ekman, Peterson, Van Veen)	Benthic invertebrates living in, or on, the sediment. Macrophytes and associated, attached organisms	Sandy or silty sediments, weed zones	Quantitative sample. Minimum disturbance to sample	Expensive. Requires winch for lowering and raising
Dredge-type	Mainly surface living benthic invertebrates	Bottom sediments of lakes and rivers	Semi-quantitative or qualitative analysis depending on sampler	Expensive. Mobile organisms avoid sampler: Natural spatial orientation of organisms disturbed.
Corer (e.g. Jenkins or made in-house)	Micro-organisms and benthic inverts	Fine sediments, usually in lakes	Discrete, quantitative	Expensive unless made in-house. Small

	living in sediment		samples possible with commercial covers	quantity of sample
Artificial substrates (e.g. glass slides plastic baskets)	Epiphytic algae, attached invertebrate species, benthic invertebrates	Open waters of rivers and lakes, weed zones, bottom substrates	Semi-quantitative compared to other methods for similar groups of organisms. Minimum disturbance to community on removal of sampler. Cheap	"Unnatural habitat", therefore not truly representative of natural communities. Positioning in water body important for successful use
Poisons (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 used.
Electro-fishing	Fish	Rivers and lake shores	Semi-quantitative. Non-destructive	Selective technique according to current used and fish size. Expensive. Safety risk to operators if not carried out carefully

Source: After Friedrich et al., 1996

Procedure

1. The net is held vertically against the river or stream bed, with the mouth facing upstream.

2. In shallow water the operator turns over the stones by hand for a defined area immediately upstream of the net. Dislodged organisms are carried into the net by the water current. In deeper water the toe or heel of the operator's boot can be used to kick the substrate in a defined area for a fixed period of time in order to dislodge organisms.

3. Stones from the defined area are examined and any attached organisms dislodged into the net.

4. The fine substrate from the sample area is also disturbed.

5 The contents of the net are gently washed into a corner of the net using flowing water and then the net turned inside out and gently shaken (and/or washed) into a sample container. Organisms clinging to the net should be removed to the sample container by hand.

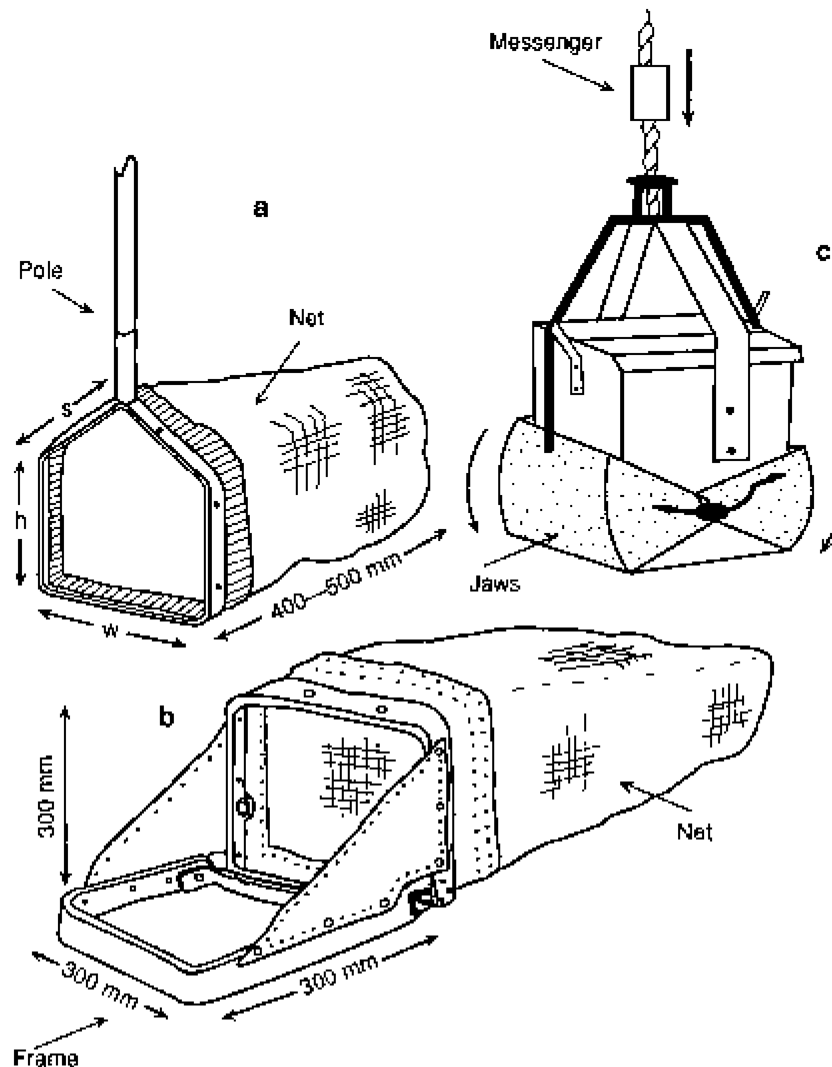
6. Ideally, organisms should be identified and enumerated live but samples can be preserved for later sorting, identification and counting (see Table 11.3 for various methods of preservation).

7. To reduce the volume of sample requiring preservation and storage, net samples can be gently washed through a 500 µm sieve and placed in a white dish containing water. The organisms can then be sorted from the stones and debris with a wide mouth pipette and placed in a labelled sample jar.

8. The net should be thoroughly washed before taking the next sample.

Quantitative samples may be obtained in water with a flow rate greater than 10 cm s^{-1} using a Surber sampler (Figure 11.3) following the same basic procedure given above for the handnet. The hinged metal frame quadrat is placed flat on the substrate with the mouth of the sample net facing upstream. The substrate within the square of the frame is stirred and lifted and organisms are dislodged into the net. The vertical side-flaps help reduce the loss of organisms around the sides of the net. When the stream bed is uneven, it may be necessary to use a foam rubber strip on the lower edge of the metal frame to close any gaps through which organisms may escape.

Figure 11.3 Various methods of sampling benthic invertebrates: a. handnet, b. Surber sampler, c. grab, e.g. Ekman grab



Slow flowing or static water, such as lakes and ponds, should be sampled using grabs, corers or dredges. Where the water is shallow and the substrate is soft, a semi-quantitative benthic sample can be obtained by pushing one end of a 25 cm diameter plastic pipe vertically into the substrate. The sediment inside the tube is then removed with a plastic beaker or fine-mesh net. In deeper water, unless access is available from a bridge or pier, a grab or corer must be used from a boat. A grab (Figure 11.3) has jaws which close beneath a known area of sediment when triggered by a messenger released down the suspension wire from the boat. The entire content of the grab represents a quantitative sample from a known area. It is usually necessary to use a corer in very fine sediments. A corer consists of a perspex cylinder (usually 15 cm diameter) with end flaps which close when triggered

automatically, or by a messenger, so that the mud and the water above it are held firmly in the tube. A dredge scrapes the surface layer collecting dislodged organisms as it is towed along.

Size of sample

The size of the sample required depends on the requirements of the ecological method and the statistical techniques which will eventually be applied to the data. Some methods require that several samples (sample units) are taken in different habitats from the sample area and pooled to create the representative sample. In other cases the single sample unit (e.g. one handnet kick sample or one grab sample) may yield insufficient numbers for statistical analysis and several units may be required for a single sample.

If the method requires an estimate of the populations of organisms from a sample site, the dimension of the sampling unit, the number of units within the sample and the location of the sampling units are all important. It is usually acceptable, for ecological surveys, to estimate the population with a precision of 20 per cent (i.e. $D = 0.2$ in the formula below). Using the results obtained from a preliminary survey, an estimate of the required number of sample units for a given precision can be calculated from:

$$n = \left(\frac{s}{D \times \bar{x}}\right)^2$$

where

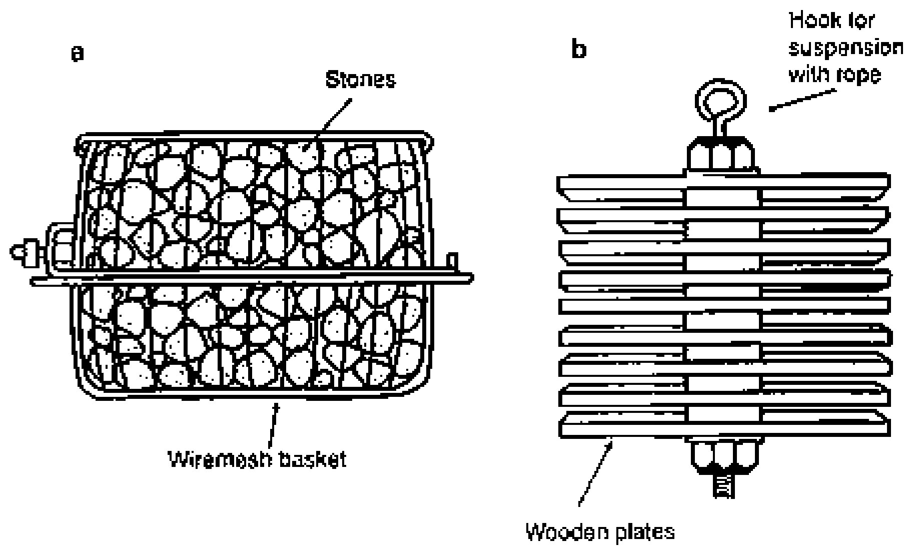
D = index of precision
$\bar{x}$ = mean
s = standard deviation
n = number of sample units

The sample units should normally be selected at random within the sample site. A grid of numbered sampling units can be allocated to the sampling site and the units selected by reference to a random number table. Further detail on the selection of sample numbers and location for statistical treatment of data is available in the companion guidebook *Water Quality Assessments*.

Artificial substrates

Where the collection of samples using traditional methods is difficult, such as in deep, fast-flowing rivers, or where it is difficult to find sites with similar physical characteristics, artificial substrates may be suitable. There are many types of artificial substrates designed for different groups of organisms. Two types of artificial substrates for invertebrates are illustrated in Figure 11.4, but other substrates include synthetic foam blocks and glass slides for micro-organisms or periphytic algae, and rope or plastic strips to imitate plants. The size of the artificial unit can be selected according to the habitat it is intended to represent and the kind of organisms that will colonise it. Some preliminary experimentation may be required to ensure that sufficient numbers of organisms can be collected from the size of artificial unit in use.

Figure 11.4 Two types of artificial substrates for sampling aquatic organisms (modified from Mason, 1981)



An artificial substrate represents a standardised sampling unit (provided it is deployed according to standardised procedures), allowing greater precision when comparing sites and reducing the natural patchiness that tends to occur within habitats. Less skill and training is required for routine collection of artificial substrates, although an experienced specialist is necessary for selecting the sample sites at which they are to be deployed.

Although artificial substrates offer several advantages, their suitability should be thoroughly tested before routine use. It takes several weeks for effective colonisation and the species composition may change if the substrate is left in place for too long. A reasonably representative community of invertebrates generally develops in about six weeks. It must be recognised that the artificial substrate will be selective for certain types of organisms and the results are not, therefore, directly comparable with other sampling techniques. However, they can be used to assess the potential of a site to support certain aquatic life purely on the basis of its water quality (and not its habitat availability).

Unfortunately, artificial substrates are subject to theft or vandalism and they may be swept away in extremely high flows.

11.2.4 Field data and habitat assessment

Habitat assessment forms an important aspect of the field work component of ecological methods because any change (natural or unnatural) in the habitat will lead to a change in the ecological balance (species, abundance and diversity of organisms present). Consequently, a field record sheet, which describes the habitat condition and indicates any signs of contamination, should be completed for each sampling site. An example of a field record sheet is given in Figure 11.5. Details relating to the specific samples taken should also be recorded in the field notebook as described in Chapter 5 (see Figure 5.7).

11.3 Measurement of chlorophyll a

A relatively simple estimate of the total mass (i.e. biomass) of algal material present can be obtained from the concentration of the photosynthetic pigment, chlorophyll. There are three main chlorophyll pigments commonly found in phytoplankton: *a, b* and *c*, but chlorophyll *a* is

the one measured most often. This pigment aids the assimilation of nutrients into cell biomass by harnessing the energy of sunlight. Its concentration is related to the quantity of cell carbon. Phytoplankton chlorophyll is one of the most commonly used biological measurements in water quality monitoring and assessments, particularly in relation to the effects of increasing nutrients or in relation to the operation of water treatment processes.

The analytical procedure for chlorophyll *a* is given in section 7.9. Water samples are usually collected from the same sites and at the same time as other measurements, using a standard water sampling technique. Chlorophyll *a* concentrations may vary with water depth depending on the penetration of light (necessary for algal photosynthesis) and whether there is sufficient turbulence to mix the algae within the water column. An integrated measure for the productive water layer (euphotic zone) can be obtained by taking depth samples and combining them or by using an integrated sampling technique, such as a hose-pipe sampler or pump sampler (see section 5.4.1). Ideally, water samples should be filtered immediately (in the field) through a glass fibre (GF/C Whatman) filter but, if necessary, they can be stored for a few hours in a cool place (out of direct sunlight) in polyethylene bottles. Zooplankton should be removed from bottled samples by passing the sample through a suitable net (e.g. 300 µm mesh).

Figure 11.5 Typical fiel record sheet for biological sampling and ecological surveys (Based on an example use by Fundaçao de Tecnologia de Saneamento Ambiental (FEEMA), Rio de Janeiro)

FIELD RECORD

Water body__________ Date__________ Time__________

Name and number of sample site__________ No.__________

Weather

Rain	None ☐	Last 24 hours ☐	Duration ______
Cloud cover	None or little ☐	Variable ☐	Extensive ☐

Water conditions

Width (m)	<1 ☐	1–2 ☐	3–5 ☐	6–10 ☐	11–20 ☐	>20 ☐
Depth (m)	<0.1 ☐	0.1–0.3 ☐	0.4–0.6 ☐	0.7–1.0 ☐	>1.0 ☐	
Currents	No turbulence ☐	Still ☐	Turbulent ☐	Strong turbulence ☐		
Estimated velocity ($m\ s^{-1}$)	<0.2 ☐	0.2–0.4 ☐	0.5–0.8 ☐	>0.8 ☐		
Turbidity	None ☐	Little ☐	Moderate ☐	Strong ☐		
Colour	Coloured ☐	Uncoloured ☐				
Odour	Absent ☐	Present ☐	Chemical ☐	H_2S ☐	Other ☐	

Substrate

Boulders	1 2 3	Silt	1 2 3	Score:
Large stones	1 2 3	Detritus	1 2 3	Rare <25% 1
Small stones	1 2 3	Mosses	1 2 3	Intermediate 25–50% 2
Filamentous algae	1 2 3	Submerged plants	1 2 3	Dominant >50% 3
Emergent plants	1 2 3	Marginal plants	1 2 3	

Habitat

% still water	Very little ☐	<25% ☐	26–50% ☐	>50% ☐
% flowing water	Very little ☐	<25% ☐	26–50% ☐	>50% ☐
Shade	None ☐	Little ☐	Moderate ☐	Heavy ☐
River protection measures	None ☐	Concrete banks ☐		Others ☐

Reducing conditions

Reducing conditions absent ☐		Reducing conditions present ☐	
Bubble-forming sediments	Yes ☐	No ☐	
Black sediment	Superficial ☐	Below sediment surface ☐	
Undersides of stones	None ☐	Partial ☐	Total ☐

Signs of pollution

Open water	None ☐	Sewage ☐	Oil ☐	Others ☐
Margins	None ☐	Sewage ☐	Oil ☐	Others ☐

Other comments__________

Observer's signature__________

Physical-chemical measurements:

pH______ Air Temp °C______

Diss. O_2 ($mg\ l^{-1}$)______ Water Temp °C______

Conductivity ($\mu S\ cm^{-1}$)______

Procedure

1. Gently mix the sample by inverting the bottle (avoiding bright light) and measure a suitable volume into a measuring cylinder (between 100 ml and 1 litre depending on the turbidity of the sample).

2. Using a gentle vacuum (i.e. a hand-operated vacuum pump), pass as much of the measured sample through a fresh GF/C filter paper as possible.

3. Add 0.2 ml of $MgCO_3$ suspension (1.0 g $MgCO_3$ in 100 ml H_2O) to the last of the sample, as it is being filtered, to preserve it on the filter.

4. Do not allow the filter to dry whilst adding the sample to the filter cup.

5. Once the sample has passed through the filter rinse the sides of the filter cup with about 50 ml of distilled water.

6. Allow the filter to dry for a few seconds and then fold it in half, with the sample folded inside.

7. Place the filter in a Petri dish or small polythene bag labelled with the sample identifier and volume filtered and, if storage is necessary, place the filter in its container in the dark, in a freezer at -20 °C. If the sample cannot be frozen until several hours later keep it cool and in the dark (note the length and conditions of storage).

8. Do not store frozen samples for more than a few months.

9. Analyse the sample as described in section 7.9.

In addition to the extraction method described above, chlorophyll can be measured by fluorescence techniques. Fluorescence measurements can be made in the field using samples immediately after removal from the water body or, with a suitable instrument, they can be made *in situ*, underwater. Extensive surveys or detailed depth profiles can be made rapidly in this way. For proper interpretation, fluorescence measured in water samples should be correlated to other variables such as extracted chlorophyll (see Figure 14.2B) or cell numbers, and instruments should be calibrated frequently against extracted chlorophyll measurements.

11.4 Physiological techniques

For the purposes of water quality monitoring, the most widely exploited physiological responses of aquatic organisms to environmental stress are production, respiration and growth rates. Most of these responses have been developed for biological monitoring under controlled conditions, such as during bioassays (see section 11.5). The growth criteria (light, nutrients, temperature) for some common freshwater algal species have been well studied and documented and several methods based on algal growth rates have now been standardised. In addition to being themselves affected by variations in water quality, phytoplankton can also directly affect water quality, particularly oxygen concentrations, turbidity and even toxicity to consumers (some algal species release chemicals which are toxic to livestock and humans when in sufficiently high concentrations). Measures of algal growth have been incorporated into some water quality management models.

For most physiological methods the results can only be considered as relative. Nevertheless, such methods are useful for monitoring large areas, along long river stretches, or for short, intensive programmes. In addition, some methods are particularly useful for monitoring the effects of effluents, where measurements can be made upstream and downstream of the discharge. An example of a relatively simple method using biological samples from the sites of interest is presented below.

Care must be taken to follow the specified procedures accurately if physiological methods are to be used successfully. Unless the method specifies a particular technique for collecting water samples, standard water samplers can be used (see section 5.5). If incubations are to be carried out *in situ*, care must be taken to avoid subjecting the samples and controls to adverse or unusual environmental conditions which might affect respiration or photosynthesis rates (such as sudden or extreme changes in temperature or light conditions).

11.4.1 Oxygen production and consumption

The net production of oxygen by phytoplankton can be used as an indicator of the activity of algae (see Figure 14.4) and of possible toxic inhibition, especially when correlated with the concentration of chlorophyll pigments. The Oxygen Production Potential has been developed as a standardised method and consists of incubating water samples containing native phytoplankton in light and dark bottles for a given time (usually 24 hours) and measuring the oxygen production. The incubations can be carried out in the bottles *in situ* (suspended in the water body) or in an incubator with heat and light. Toxic inhibition should be suspected where the Oxygen Production Potential decreases per unit of algal biomass (measured as chlorophyll). The Oxygen Production Potential can also be expressed as the equivalent concentration of chlorophyll required to generate 10 mg l^{-1} of oxygen.

An alternative method is to measure oxygen consumption resulting from respiration by bacteria in the test water samples. Substrates which stimulate bacterial growth (e.g. peptone or glucose) are added to the water samples and the concentration of oxygen after incubation is compared with controls without growth stimulators. Greater oxygen consumption in the test samples suggests bacterial activity is normal, but if oxygen consumption ceases or is very low, the samples may contain a substance which is toxic to the bacteria.

11.5 Controlled biotests

Bioassay methods can be used to reveal or confirm the presence of toxic conditions in water bodies as well as to provide information on the toxicity of effluents. Bioassay methods can be used to demonstrate the presence of “unknown” contaminants, to locate the position of diffuse or point discharges of contaminants or to monitor the dispersion of known toxic discharges. In addition, such methods are useful for evaluating persistence and the combined effects of several contaminants or effluents.

Toxicity tests and bioassays can be used to evaluate the necessity or urgency for chemical analysis when many samples have been taken, for example, following an accidental pollution incident. A rapid evaluation of toxicity can highlight the samples requiring immediate chemical analysis, thereby focusing the attention of the chemical analysis laboratory on samples from areas where the greatest environmental effects, and possible risks to water users, may be found.

Table 11.5 Examples of standardised bioassay techniques

Organism	Response	Exposure time	Standard method
Bacteria			
Pseudomonas putida	Inhibition of oxygen consumption	30 minutes	DIN 38 412 Part 8 (1991) DIN 38 412 Part 27 (1993)
Vibrio fischeri[1]	Inhibition of luminescence	30 minutes	DIN 38 312 Part 34 (1991) DIN 38 312 Part 341 (1993)
Invertebrates			
Daphnia magna	Immobilisation	24 hours	DIN 38 412 Part 11 (1982) DIN 38 412 Part 30 (1989) OECD 202 Part I (1984)
	Reproduction	14 days	OECD 202 Part II (1993)
Fish			
Brachydanio rerio	Death	1-2 days	ISO 7346/1/2/3 (1984) OECD 203 (1992)
	Sub-lethal effects	14 days	OECD 204 (1984)
	Death	28 days	DIN 38 412 Part 15 (1982) DIN 38 412 Part 20 (1981) DIN 38 412 Part 31 (1989)
Oncorhynchus mykiss	Sub-lethal effects	14 days	OECD 204 (1984)
Algae			
Scenedesmus subspicatus	Growth rate, reproduction	3 days	ISO 8692 (1989) DIN 38 412 Part 33 (1991)
Selenastrum capricornutum	Growth rate, reproduction	3 days	ISO 8692 (1989) OECD 201 (1984)

[1] *Phytobacterium phosphoreum*

Acute toxicity is usually indicated by death and standard laboratory toxicity tests use this response to assess the lethal concentration of a sample or compound. Bioassays usually measure more subtle, sub-lethal effects in organisms under defined conditions (for a particular temperature or time interval). Bioassays can be carried out *in situ* in the water body, or in the laboratory using samples of water collected from the field. When compared with acute tests, sub-lethal methods are generally found to be more sensitive to the dilutions of contaminants expected in water bodies after discharge and dispersion. Many sub-lethal responses can be detected by physiological measurements (see also previous section), such as growth rates or oxygen production, while others can be manifest by behavioural, biochemical or mutagenic changes.

Test organisms can be exposed *in situ* and the response measured at regular time intervals or they can be placed at selected sites over a study area and the results evaluated from all sites for the same time interval. This is known as active biomonitoring. Responses such as

mortality, growth and reproduction are commonly used in these situations. Some physiological or behavioural responses, such as swimming activity, algal fluorescence or oxygen consumption, are suitable for continuous monitoring in early warning systems (see below). Physical and chemical variables should be monitored simultaneously during any *in situ* exposure in order to help eliminate effects on the test organisms other than those due to the contaminant under investigation.

It is very important, when conducting or developing bioassays, to select organisms which are compatible with the water samples being tested, i.e. organisms which tolerate the normal physical and chemical conditions of the test water (such as salinity, temperature, hardness). There are now many standardised bioassays using widely available organisms which are easily maintained in the laboratory (Table 11.5). However, the relevance of the chosen tests to the individual water body and its associated water quality problems needs to be carefully evaluated before use. For tests carried out *in situ* it is important to use indigenous organisms.

The following section describes a rapid test for determining the effects on the microcrustacean organism *Daphnia magna* resulting from chemicals or substances in a water sample or from municipal or industrial wastewater samples. The precise details of other standardised tests are available in the relevant documentation (see section 11.9). Some general guidance on site selection and water sampling is given in section 11.7.

11.5.1 *Daphnia magna* immobilisation test

Daphnia magna Straus are members of the zooplankton of still waters and obtain their nutrition by filter feeding on particulate organic matter. The effects of a water sample or an effluent on the *Daphnia* can be determined and expressed as the concentration at the start of the test which results in 50 per cent of the test organisms becoming immobile and incapable of swimming during the 24 hours of the test period. This concentration is known as the effective inhibitory concentration and is designated as the 24h-$EC50_i$. If it is not possible to record the 24h-$EC50_i$, or even a 48h-$EC50_i$, useful information can be gained from the lowest concentration tested which immobilises all the *Daphnia* and the highest concentration tested which does not immobilise any of the organisms.

A preliminary test using a single series of concentrations can be carried out which determines the range of concentrations for use in the final toxicity test and gives an approximate value of the 24h-$EC50_i$ (or, where appropriate, the 48h-$EC50_i$). When the approximate value obtained in the preliminary test is not adequate a definitive test is carried out. This allows calculation of the 24h-$EC50_i$, the 48h-$EC50_i$ and determination of the concentrations corresponding to 0 per cent and 100 per cent immobilisation. The range of concentrations of the test solution must be chosen to give at least three percentages of immobilisation between 10 per cent and 90 per cent.

The test should be carried out on samples within 6 hours of collection whenever possible. Samples can be stored for up to 48 hours if cooled to 4°C immediately on collection, or they can be frozen and used for testing within 2 months.

Apparatus

√ pH meter.

√ Oxygen meter.

√ Glass beakers, 50-ml and 2,000-ml, washed and rinsed first with distilled water and then with dilution water.

√ Culture tubes, 18 mm diameter, 180 mm high, washed and rinsed first with distilled water and then with dilution water.

√ Petri dishes, washed and rinsed first with distilled water and then with dilution water.

√ Matt black surface.

√ Sieves, nylon meshes of 0.65 mm and 0.20 mm apertures.

√ Graduated flasks, 100-ml and 1,000-ml.

√ Graduated pipettes, 1, 10 and 25 ml.

√ Pasteur pipettes.

Consumables

Analytical reagent grade chemicals and pure (e.g. deionised or distilled) water must be used.

√ Calcium chloride solution: dissolve 11.76 g of calcium chloride dihydrate, $CaCl_2.2H_2O$, in water and make up to 1,000 ml.

√ Magnesium sulphate solution: dissolve 4.93 g magnesium sulphate heptahydrate, $MgSO_4.7H_2O$, in water and make up to 1,000 ml.

√ Sodium bicarbonate solution: dissolve 2.59 g of sodium bicarbonate, $NaHCO_3$, in water and make up to 1,000 ml.

√ Potassium chloride solution: dissolve 0.23 g of potassium chloride, KCl, in water and make up to 1,000 ml.

√ Dilution water prepared by transferring 25 ml, by pipette, from each of the above four solutions into a graduated flask and making the volume up to 1,000 ml. Dilution water should be made with water of maximum conductivity 10 µS cm^{-1}. The final pH must be 7.8 ± 0.2 and hardness 250 mg l^{-1} (as $CaCO_3$) with a molar Ca:Mg ratio close to 4:1. The dissolved oxygen concentration must be greater than 7 mg l^{-1}. Natural water of similar pH and hardness may be used for culture. Dilution water must be aerated until the pH has stabilised and the dissolved oxygen concentration has reached saturation. The pH can be adjusted with sodium hydroxide or hydrochloric acid.

√ Potassium dichromate, $K_2Cr_2O_7$, of analytical grade.

√ *Daphnia magna* Straus of at least third generation. A clone culture can be purchased or raised in the laboratory.

√ Food for *Daphnia*, e.g. single-celled green algae.

Procedure

1. Start the culture by adding no more than 100 adult *Daphnia* per litre of culture water in large glass beakers. Add a quantity of food which can be consumed within a few days (the water in the beaker will go from green to clear as the algae are consumed).

2. Keep the culture either in the dark or under a 16 h/8 h light/dark photoperiod at 20 ± 2°C and change the culture water once or twice a week. When changing the water separate adults from young by passing the culture through a fine and a coarse mesh sieve. The adults are retained on the coarse sieve and the younger ones on the fine sieve.

3. To obtain *Daphnia* less than 24 hours old for the test, select gravid females from the adults retained on the coarse sieve and transfer into a fresh culture vessel. Collect newly released neonates with a fine sieve within 24 hours.

4. Using dilution water, prepare the desired dilutions or concentrations of the test sample or effluent immediately before conducting the test.

5. Pour 10 ml of each test solution into a series of culture tubes or 20 ml into a series of beakers. For samples with a high oxygen consumption use 20 ml volumes in Petri dishes.

6. Add no more than 20 *Daphnia* to each container of test concentration or dilution (a maximum of 5 *Daphnia* per 10 ml of test solution is recommended). For each test series prepare one control of dilution water only (using the same volume of solution and number of *Daphnia* as used for the test solutions).

7. Place control and test samples in the dark or 18 h/8 h light/dark at 20±2 °C for 24 hours without food.

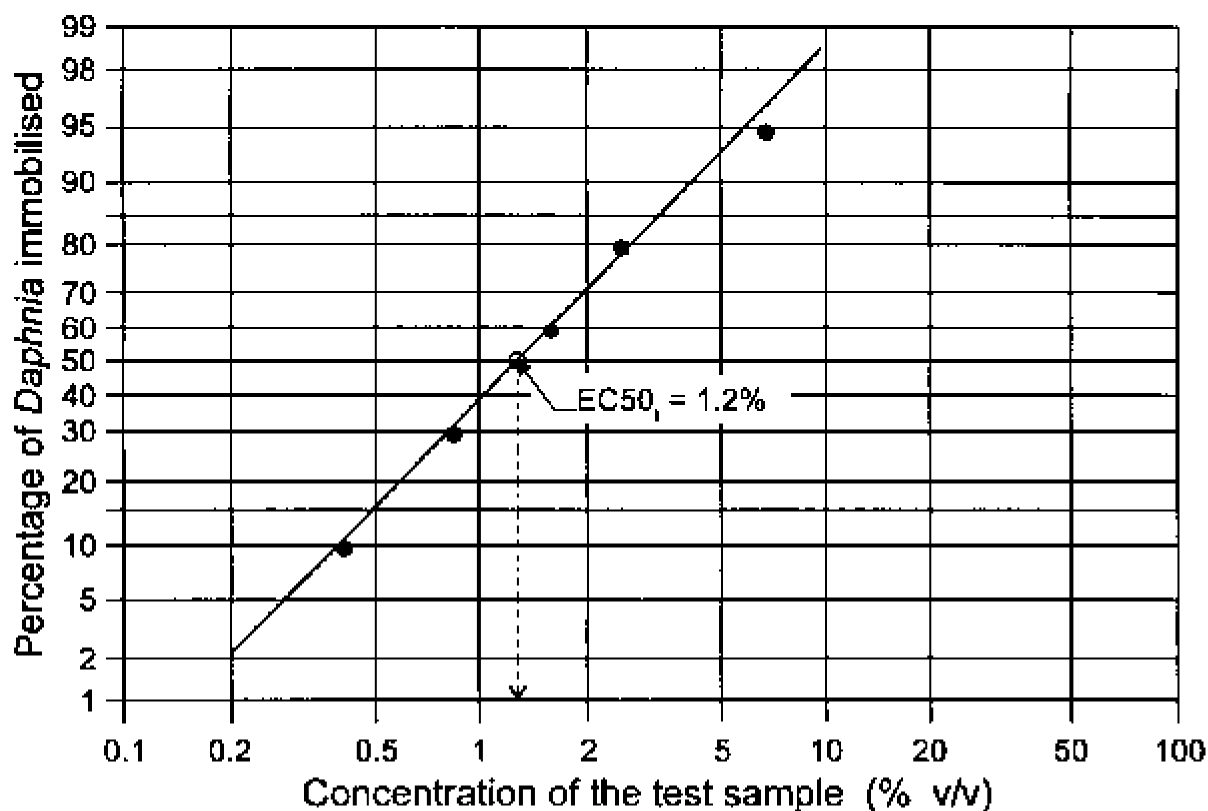
8. After 24 hours gently agitate the liquid in each beaker for 15 seconds and place it on a matt black surface. Count and record the number of *Daphnia* in each beaker which do not immediately exhibit swimming behaviour when the water is agitated.

9. Determine the concentration range giving 0 per cent to 100 per cent immobilisation and note abnormalities in the behaviour of the *Daphnia.*

10. Record the dissolved oxygen concentration in the test container with the solution of lowest concentration at which all the *Daphnia* were immobilised.

11. Periodically determine the 24h-$EC50_i$ (following the procedure above) of the potassium dichromate reference solution (by making up a range of concentrations with dilution water). This verifies the sensitivity of the *Daphnia*. If the 24h-$EC50_i$ falls outside the concentration range 0.6-1.7 mg l^{-1} the application of the test procedure should be checked, as well as the culture technique for the *Daphnia*. A new strain of *Daphnia magna* may be necessary.

Figure 11.6 Graphical representation of the results from an acute *Daphnia* test from which the $EC50_i$ value can be interpolated.



Evaluation and expression of results

Calculate the percentage immobilisation for each test concentration. The $EC50_i$ value can then be obtained by calculation or by interpolation from a graph such as that in Figure 11.6, where the concentration or dilution of the test sample is plotted on the x-axis (logarithmic values on probability paper can be used) and the corresponding percentage of the *Daphnia* incapable of swimming are plotted on the y-axis. The highest concentration of the sample in which all *Daphnia* remained capable of swimming (0 per cent immobilisation) and the lowest concentration at which all *Daphnia* were incapable of swimming (100 per cent immobilisation) should also be recorded, particularly if data are insufficient to calculate the $24h\text{-}EC50_i$.

The test is only valid a) if less than 10 per cent of the *Daphnia* in the control are incapable of swimming, b) if the $EC50_i$ value of the potassium dichromate lies in the range 0.6-1.7 mg l^{-1} and c) if the dissolved oxygen concentration at the end of the test was $\geq$ 2 mg l^{-1}.

Results are reported as a percentage dilution in the case of test samples or as mg l^{-1} for specified chemical substances. The $24h\text{-}EC50_i$ and the concentrations resulting in 0per cent and 100 per cent immobilisation should be quoted, together with any other relevant information.

11.5.2 Early warning methods

Biological early warning systems (also referred to as biomonitors and dynamic tests) rely on a biological response in the test organisms placed *in situ* triggering further action when defined thresholds (expressed in terms of the biological response) are exceeded. The further action usually takes the form of a more detailed investigation of the causes, including

chemical monitoring. If these systems are used close to important water intakes, such as for major drinking water supplies, the response can be used to trigger the temporary shut down of the intake while further investigations are carried out. When used close to major effluent discharges, the biological responses can be used to signal a sudden change in the nature of the discharge, such as may occur due to a treatment plant malfunction. Rapid measures to reduce environmental effects can then be taken without waiting for extensive chemical monitoring results.

There are many commercially available biological early warning systems and some can be fairly easily constructed to suit specific requirements. They are, however, expensive to set-up and to run efficiently. The most common organisms employed are fish, the crustacean *Daphnia* sp., algae and bacteria. The basic principle usually involves diverting water from the water body of interest, as a continuous flow, through special tanks containing the test organisms. The response of the organisms is continuously measured by an appropriate sensor device and recorded, for example by computer or by paper and pen recorder. Some semi-continuous flow tests take water into the tank containing the test organisms and then close the intake for a defined period during which the responses are recorded. The test water is then renewed for a new test cycle.

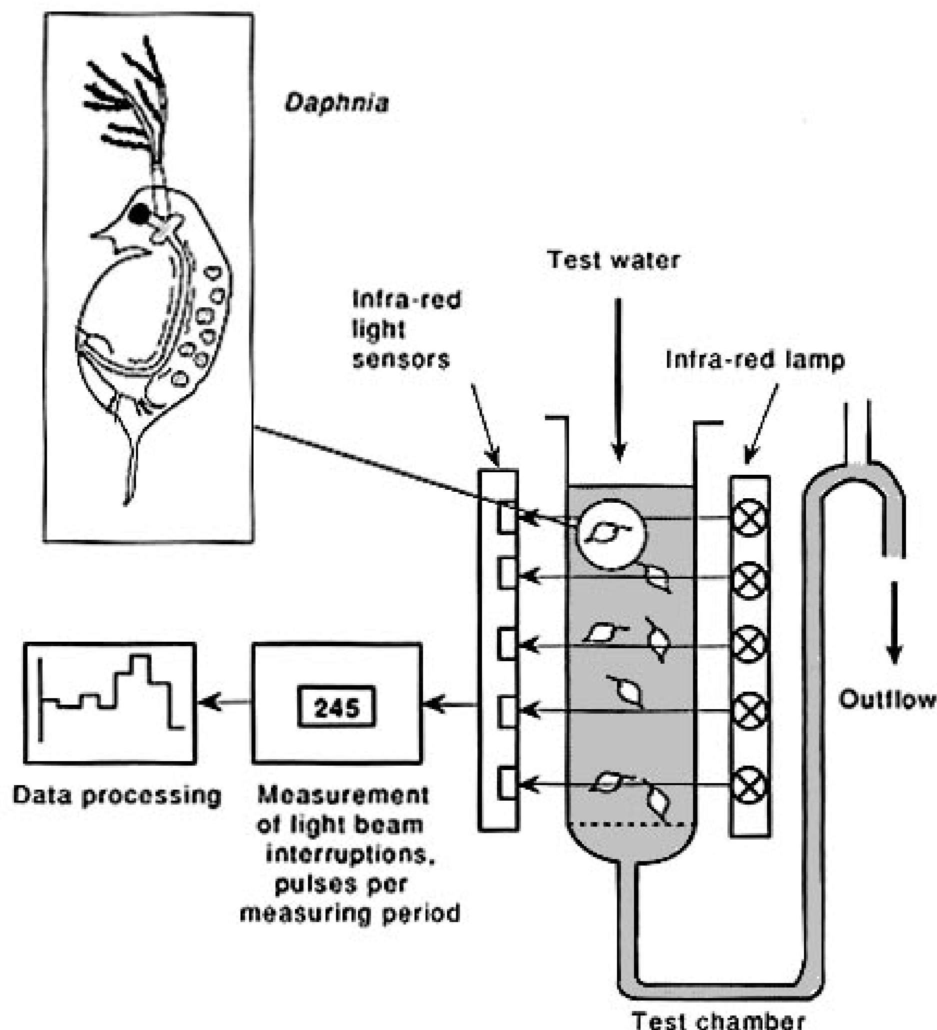
Certain organisms have been shown to be particularly suitable for indicating the presence (although not the nature or concentration) of certain chemicals. Fish and *Daphnia* are able to indicate the presence of many insecticides, and algae are able to indicate the presence of herbicides (through photosynthetic inhibition). In order to act as a general early warning mechanism for unspecified contaminants, several tests using organisms of different trophic levels should be used simultaneously.

Biomonitors using algae rely on detecting relative changes in *in-vivo* chlorophyll fluorescence at 685 nm. High energy lights (435 nm) are used to activate the chlorophyll *a* molecules of the algae and the energy which is not used for photosynthesis is emitted as fluorescence. Damaged algae (for example by the presence of toxic compounds) show increased fluorescence.

Methods using bacteria are based on the measurement of oxygen depletion in the test tank arising from normal bacterial respiration. If the oxygen concentrations are not reduced in the test water after contact with the bacteria the test sample is assumed to have had a toxic effect on the bacteria. The test water is first enriched with oxygen and oxygen electrodes are used to measure the initial oxygen concentration in the test sample and the residual oxygen in the contact tank containing the test bacteria.

The planktonic crustacean *Daphnia* sp. is an important member of many aquatic communities, where it forms a link in the food chain by grazing on algae and forming a food source for small and juvenile fish. The difference between the swimming activity of *Daphnia* in a control tank and a tank receiving test water is used as a measurable response to the presence of contaminants causing inhibition of movement. The swimming activity is measured by an array of light beams and light sensors (Figure 11.7). This dynamic *Daphnia* test has been shown to be highly sensitive and reliable over many years of operation.

Figure 11.7 A biological early warning system using *Daphnia* in a continuous flow-through chamber with light sensors to detect variations in motility (Details provided by the State Agency for Water and Waste, North-Rhine/Westphalia, Germany)



Although the use of fish has the advantage that the test organisms are vertebrates (and hence closer to humans in their physiological and biochemical responses than other test organisms), their biological responses which are sufficiently sensitive to be used in early warning tests for unknown contaminants require complex measurement techniques. One approach uses a computer-controlled image processing system to detect and analyse the behaviour in a pair of continuous flow-through tanks each containing a shoal of six small fish. The activity of the fish in each tank is recorded by two video cameras and six behavioural parameters are analysed by the computer for each tank: i.e. motility, irregularity of swimming velocity, number of turnings, swimming height, mean horizontal position and shoaling behaviour. The results are compared statistically with “normal” behaviour patterns.

11.6 Contaminants in biological tissues

Many organisms have been found to accumulate certain contaminants in their tissues during their life time without detectable effects on normal physiological functions. This phenomenon is often known as bioaccumulation. The contaminants are effectively detoxified and/or tolerated within the organism, often as a result of binding into particular tissues in the body. Some organisms accumulate particular contaminants in this way until a critical body burden

is reached which then triggers a physiological effect, other organisms regulate the body burden by excreting the contaminant. In some organisms, a sudden change in metabolic pattern (such as during breeding) causes the remobilisation of the accumulated contaminants, subsequently producing adverse physiological effects. The transfer of contaminants from one trophic level to another, through ingestion of organisms which have already bio- accumulated a contaminant, is known as food-chain transfer. This may lead to organisms higher in the food chain accumulating contaminants to concentrations much higher than occur in the ambient surroundings.

If the correlation between the concentration of a contaminant in a water body and the concentration in the tissues of an aquatic organism is good, the organism may be used for chemical monitoring of the contaminant in the water body as an alternative monitoring medium to water or particulate matter. Biological tissues sometimes have the advantage that the contaminant concentrations being analysed are much higher than in water or particulate matter, thus requiring lower detection limits and, possibly, less sophisticated analytical techniques. In addition, biological tissues can be bulked together to give a greater total concentration.

At present there are few species which can be used world-wide for chemical monitoring, although many indigenous organisms have been tested and found suitable for local use. Analysis of organisms such as fish and shellfish, which are consumed by human populations, provides information of direct relevance to human health. Active monitoring of the distributions of contaminant concentrations can be carried out by deliberately placing organisms at specified locations in water bodies and collecting them for analysis after suitable time intervals have elapsed during which bioaccumulation may have occurred. Examples of the use of organisms in chemical monitoring are given in the companion guidebook *Water Quality Assessments*.

11.6.1 Organism selection

Organisms suitable for chemical monitoring should be immobile (such as bivalve molluscs) or should remain or should be restrained (e.g. in a cage) within the area which is being studied so that that they are not reflecting the accumulation of contaminants from another area from which they may have migrated. Plants and animals have been found to be useful for monitoring contamination by metals, organic chemicals and radioisotopes, and many examples of organisms and methods can be found in the published literature.

When investigating suitable local organisms certain criteria should be tested and met:

- The organism should accumulate the contaminant of interest when exposed to the environmental concentrations present without experiencing lethal toxic effects.

- There should be a simple correlation between the concentration of the contaminant in the organism and the average concentration in the water body.

- Bioaccumulation should reach concentrations which allow direct analysis of organism tissues.

- The organisms should be abundant in, and representative of, the water body being monitored.

- The organism should be easily sampled.

• The organism should survive in the laboratory long enough for contaminant uptake studies to be performed.

Assuming all these criteria can be met for the chosen organism, it is also important that they are fulfilled at each sampling site under all the environmental conditions that may be encountered during the monitoring programme. In order to make valid comparisons between sites in a water body using this technique, the correlation between contaminant concentrations in the biological tissues and the water body must be the same for all sampling sites.

11.6.2 Procedures

Most chemical analyses of biological tissues are performed using standard techniques, such as atomic absorption spectrophotometry (AAS) with flame or graphite furnace for metals (see section 8.1), gas chromatography-mass spectrometry for organic micropollutants (see section 8.2) or high pressure liquid chromatography with fluorescence for polyaromatic hydrocarbons. Acid digests of the biological tissues are used for metal analyses and organic solvent extracts are used for organic contaminants. Usually between 10 g and 15 g of dry tissue is required and results are expressed as $\mu g\ g^{-1}$ dry weight or $mg\ kg^{-1}$ wet weight or dry weight. For contaminants with a strong affinity for lipids the results may be expressed as $mg\ kg^{-1}$ fat content.

The handling of tissues for chemical analysis requires the same degree of cleanliness as for water sample analysis in order to avoid contamination. Great care must be taken during all stages of sample handling, including collection in the field, transportation to the laboratory and during laboratory preparation. For example, where tissues are to be analysed for metals, only acid washed plastic or glass samplers, containers and instruments should be used and all procedures, such as dissecting, drying and weighing, should be carried out in a clean, preferably dust-free, environment. At least duplicate analyses should be made for each sample and sample blanks should be prepared with each batch of tissues in order to check for contamination (see Chapter 9).

There are many detailed descriptions, available in the published literature and specialised texts, of satisfactory techniques which have been developed for common contaminants in many different biological tissues. The basic procedure for metal analysis is outlined here as a basis for developing, or selecting methods, appropriate to the specific objectives of a monitoring programme.

Procedure

1. Select organisms of a similar species, sex, size (weight or length) and stage of life cycle. If whole organisms are to be analysed a period of depuration in uncontaminated water may be necessary (during which the organisms flush out the contents of their guts).

2. Sampled organisms should be transported and/or stored in specially cleaned containers appropriate to the future analyses, e.g. acid-cleaned plastic pots or new polythene bags. If analyses will not be performed for several days or weeks, samples (whole organisms or dissected tissues) should be frozen immediately and stored at -20 °C.

3. Weigh whole organisms or selected tissues if wet weight is to be used or oven- or freeze-dry to constant weight and then weigh if dry weights are to be used.

4. Add a small volume of high grade concentrated nitric acid to the dry tissue in an appropriately cleaned container (e.g. glass test tube with reflux bulb or PTFE beakers with

covers). If the whole sample is too large for digestion about 10-15 g of dry tissue should be weighed out.

5. Heat the samples gently (e.g. on a hot plate at 70 °C) until digestion of the tissue is complete and no residue remains. The digest should be clear and not cloudy.

6. Make up the digest to a known volume (e.g. 50 ml) with analytical-grade water.

7. Analyse the digest, or suitable dilutions of it, using an appropriate technique (such as AAS, see section 8.1) and calculate the concentration of the metal in terms of the wet or dry weight of tissue (mg kg^{-1} or µg kg^{-1}). Allowance must be made for any measurements obtained from the blank digests.

11.7 Site selection and sampling frequency

Selection of sampling site location and sampling frequency depend on the objectives of the monitoring programme, together with any special requirements of the biological techniques being used. Consideration should be given to sites where other physical and chemical measurements are made. The criteria for site selection are, therefore, fundamentally the same as has been discussed in Chapter 3. Reference can be made to any earlier biological monitoring results as an aid to site selection.

Most of the general principles for site selection in relation to the programme objectives described in Chapter 3 are applicable for ecological methods. However, the suitability of the site for the anticipated sampling method also has to be tested. It is preferable that ecological samples are taken at the same sites as hydrological measurements and samples for physical and chemical analysis, in order to have the maximum information possible available for interpretation of any ecological effects observed.

Typically, ecological methods are used for long-term evaluation of biological water quality by sampling once a year in the same place and at the same time of year. Intensive surveys to assess baseline ecological quality may be conducted several times a year in the first instance in order to determine seasonal variations in the organisms present. The life cycles of organisms and the ability for their populations to recover from sampling activities which totally remove all species, must be taken into consideration.

Since many bioassay and physiological techniques rely on detecting a biological response which differs from normal, it is usually essential to find additional sites which can act as controls, i.e. the water is uncontaminated and the organisms found there are not subject to the same environmental stress (such as upstream of discharges to a river). Recovery sites (beyond the mixing zone of contaminants) should also be selected, as well as sites which cover the full range of the suspected pollution gradient, e.g. at intervals downstream of a discharge to a river, or in a grid radiating outwards from a point source in a lake. Water samples for bioassay techniques should be taken, whenever possible, when the environmental dilutions of contaminant inputs are at their lowest, such as during low flow periods in rivers.

Discrete or composite water samples can be taken with most water samplers as described in section 5.5. The frequency of sampling is determined by the objectives, as for all kinds of monitoring. Where accidental contamination is being monitored, the frequency of sampling will be high (e.g. daily) and determined by the severity and duration of the effects, although the activity might only continue for a total period of weeks. Early warning systems are usually in operation, *in situ,* continuously.

The same principles apply to sampling biological materials for chemical analysis as for taking water samples for similar analyses. For passive monitoring, collection of organisms of a particular growth stage or period of the life cycle may require sampling at a particular time of year. Active monitoring requires placement of the organisms in carefully selected control and exposure sites which are readily accessible and environmentally suitable for the test organisms.

11.8 Quality assurance

The general principles of quality assurance described in Chapter 9 should also be applied to all biological methods. The precise details of the methods adopted for a monitoring programme should be recorded in the standard operating procedures and made available to all personnel involved. This includes the details of field sampling techniques (e.g. length of time required for an invertebrate kick sample, precise orientation of an artificial substrate in relation to flow direction, etc.) as well as the laboratory-based procedures. This ensures that all personnel use exactly the same procedures for a particular monitoring programme, regardless of techniques they may have used elsewhere. Relevant observations should be recorded at all steps in the procedure, from the field record to the laboratory worksheets and the final calculations, so that discrepancies or unusual results can be checked back against all the raw data. Any deviations from the standard operating procedure should be recorded.

In order to compare the results of biological monitoring obtained by different laboratories or organisations, it is necessary to use generally accepted and standardised methods. With the increasing interest in, and use of, biological approaches an increasing number of methods are being tested and standardised at national and international level. The precision of the methods should be known and, where possible, acceptable limits of accuracy should be set according to the requirements of the monitoring programme rather than defined by the easiest method available.

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